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WITNESS my hand this
Tenth day of April 2001

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A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

**"A METHOD OF MODULATING CELL SURVIVAL AND
REAGENTS USEFUL FOR SAME"**

The invention is described in the following statement:

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A METHOD OF MODULATING CELL SURVIVAL AND REAGENTS USEFUL FOR SAME

5 FIELD OF THE INVENTION

The present invention relates generally to a method for modulating cell survival. Modulation of cell survival includes inducing, enhancing or otherwise promoting cell survival such as the survival of neuronal cells as well as facilitating cell death such as the death of targetted cancer cells. The modulation of cell survival is mediated by a region identified on the p75
10 neurotrophin receptor (p75^{NTR}) required for death signalling. The present invention further provides genetic molecules which encode the death signalling region of p75^{NTR} which are useful in antagonising death signal function as well as promoting cell death when expressed in targetted cells. The present invention also contemplates recombinant peptides, polypeptides and proteins as well as chemical equivalents, derivatives and homologues thereof which
15 comprise the death signalling portion of p75^{NTR}.

BACKGROUND OF THE INVENTION

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description.

20

The increasing sophistication of recombinant DNA technology is greatly facilitating research and development in the medical and allied health fields. This is particularly the case in the development of recombinant cytokines and growth factors for use in the treatment of diabetes, aquired immunodeficiency syndrome (AIDS) and a number of cancers.

25

However, despite this developing knowledge of cytokine and growth factor effector molecules, their full exploitation requires an understanding of the corresponding cellular receptors and the complex biochemical and physiological signalling pathways initiated following interaction with ligands or following other stimulation such as disease, receptor
30 aggregation or trauma.

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A number of soluble trophic factors have been shown to exhibit an effect on neuronal survival *in vivo*. Many of these factors act directly on the developing neuron within, for example, the dorsal root ganglia (DRG). One factor of particular importance is nerve growth factor (NGF) [1]. The p75 neurotrophin receptor (hereinafter referred to as "p75^{NTR}"), which is
5 capable of complexing with trk growth factor receptors, is required for high affinity NGF binding and survival signalling. Although NGF has been proposed as a potential therapeutic molecule to promote survival of neurons, NGF is a multifunctional molecule and its pleiotrophy may adversely effect a range of non-neuronal cells.

10 p75^{NTR} is also multifunctional. It has now been shown that p75^{NTR} is capable of acting as a death receptor. Elevated p75^{NTR} expression results in increased cell death *in vitro* and *in vivo* [2-4]. Furthermore, down-regulation of p75^{NTR} prevents neuronal death after growth-factor withdrawal or axotomy [5, 6]. Consistent with the dual functions of p75^{NTR}, mice with deleted p75^{NTR} genes have a dramatic reduction of NGF dependent neurons, such as dorsal
15 root ganglia, but increased numbers of other neuron populations (sympathetic and basal forebrain neurons) suggesting lack of naturally occurring cell death [7, 8]. p75^{NTR} is also implicated in mediating death of neuronal, oligodendrocytes and Schwann cells [8, 9].

p75^{NTR} is a member of the tumor necrosis factor (TNF) receptor/Fas superfamily, showing
20 homology not only to the extracellular ligand binding domain but also to a cytoplasmic motif known as the "death domain", so termed because of the cytotoxic actions of proteins containing the domain [9].

There is an accumulating body of evidence which suggests that p75^{NTR} is involved in
25 mediating cell death in a variety of degenerative diseases. During adulthood, p75^{NTR} expression is down-regulated in most brain areas but is rapidly induced in ischemia (stroke) and results in transient increased p75^{NTR} expression and apoptosis, as do both peripheral and motor nerve lesions [10-12]. p75^{NTR} is also up regulated in patients with MND [13], and in experimental allergic encephalomyelitis (a model of multiple sclerosis; [14]). Intriguingly, in
30 the basal forebrain and hippocampus, areas involved in learning and memory, p75^{NTR} is highly expressed in aged rodents and in Alzheimer's patients, where extensive neuronal death

is occurring [15, 16]. These data suggest that p75^{NTR} is involved not only in normal developmental cell death, but may mediate the cell death occurring after injury or in neurodegenerative disease.

- 5 In work leading up to the present invention, the inventors sought to elucidate the region on p75^{NTR} which mediates death signalling. The inventors surprisingly determined that the death signal is not the cytoplasmic motif known as the death domain [9] but is a region adjacent the membrane domain on p75^{NTR}. The identification of this region provides for an opportunity to modulate cell survival by antagonising the death signalling region or promoting apoptosis by
- 10 providing cells with the genetic material to express the death signalling region adjacent, proximal or otherwise juxtaposed or associated with the membrane or to express the death signalling region in multimeric form.

SUMMARY OF THE INVENTION

- 15 Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.
- 20 Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide and amino acid sequences referred to in the specification are defined following the bibliography.

- One aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides which encode an amino
- 25 acid sequence which is capable of signalling, inducing or otherwise facilitating the death of a cell in which said amino acid sequence is adjacent, proximal or otherwise juxtaposed to the membrane of said cell or said amino acid sequence is in multimeric form.

- Another aspect of the present invention is directed to a nucleic acid molecule comprising a
- 30 sequence of nucleotides or complementary sequence of nucleotides which encodes a peptide, polypeptide or protein capable of signalling, inducing or otherwise facilitating death of a cell

in which it is expressed wherein said peptide, polypeptide or protein comprises a membrane associating portion and/or a multimer-forming portion and a portion which corresponds to all or part of the cytoplasmic region of p75^{NTR} or a functional equivalent, derivative or homologue thereof.

5

Yet another aspect of the present invention contemplates homologues, analogues and derivatives of a nucleic acid molecule which encodes a peptide, polypeptide or protein which is capable of signalling inducing or otherwise facilitating death of a cell in which it is expressed wherein said peptide, polypeptide or protein comprises a membrane associating
10 portion and/or a multimer-forming portion and a portion which corresponds to all or part of the cytoplasmic region of p75^{NTR} or a functional equivalent, derivative or homologue thereof.

Still another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence which is substantially as set forth
15 in SEQ ID NO:3 or is a nucleotide sequence capable of hybridising thereto under low stringency conditions at 42 °C or is a nucleotide sequence having at least 60% identity thereto.

Still yet another aspect of the present invention contemplates a nucleic acid molecule
20 comprising a nucleotide sequence or a complementary form thereof, which nucleotide sequence encodes an amino acid sequence substantially as set forth in SEQ ID NO:4 or a derivative, homologue or chemical equivalent thereof or an amino acid sequence having at least 60% identity thereto.

25 Even yet another aspect of the present invention provides a genetic construct comprising an isolated nucleic acid molecule which comprises a sequence of nucleotides which corresponds or is complementary to a death signal region from p75^{NTR} or a homologue, analogue or derivative thereof.

30 Another aspect of the present invention contemplates an isolated peptide, polypeptide or protein comprising the cytoplasmic region of p75^{NTR} which signals, induces or otherwise

- 5 -

facilitates cell death when said peptide, polypeptide or protein is adjacent, proximal or otherwise juxtaposed to a membrane-associating region such as from p75^{NTR} or other membrane molecule and/or said peptide, polypeptide or protein is capable of forming multimers or a derivative, homologue, chemical equivalent or analogue of said peptide, polypeptide or protein. This aspect of the present invention does not extend to the full length p75^{NTR}.

Still another aspect of the present invention contemplates a method for inhibiting, reducing or otherwise antagonising a p75^{NTR}-mediated death signal in a neuronal cell, said method comprising introducing a nucleic acid molecule capable of being expressed to an expression product which corresponds to a non-membrane associated form of the p75^{NTR} death signal region or a derivative, functional equivalent or homologue thereof.

Even still another aspect of the present invention provides a biological composition comprising a genetic molecule capable of being expressed into a p75^{NTR} death signal antagonist or a p75^{NTR} death signal.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a diagrammatic representation showing plasmid constructs with and without the death signalling region. The black region is the putative "death domain" [9] but which is not directly involved in p75^{NTR} mediated cell death.

Figure 2 is a graphical representation showing survival of DRG neurons 17 hours after microinfection and cultured in LIF. The data show that the amino acid domain juxtaposed to the membrane is required for death signalling rather than the putative "death domain" [9].

Figure 3 is a graphical representation showing DRG survival 16 hours after microinjection and cultured in LIF. The data show that over 90% of cells die when expressing the death signal linked to the membrane.

Figure 4 is a graphical representation showing DRG survival 20 hours after microinjection and cultured in LIF. These data show that when the death signal is not associated with the membrane, that the ability to induce death is removed.

- 5 **Figure 5** is a graphical representation showing that the C35 protein (i.e. p75^{NTR} death signal region) inhibits death signalling mediated by p75^{NTR}.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

- The present invention arose in part following an investigation of the neurotrophin receptor, p75^{NTR}, in its capacity as a death signalling protein. Although the p75^{NTR} molecule comprises a putative death domain [9], in accordance with the present invention, this death domain is not directly associated with p75^{NTR}-mediated cell death. Rather, a region adjacent, proximal or otherwise juxtaposed to the membrane domain of p75^{NTR} is required for cell death.
- 15 Accordingly, one aspect of the present invention provides an isolated nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides which encode an amino acid sequence which is capable of signalling, inducing or otherwise facilitating the death of a cell in which said amino acid sequence is adjacent, proximal or otherwise juxtaposed to the membrane of said cell or said amino acid sequence is in
- 20 multimeric form.

- Reference herein to the signalling, inducing or otherwise facilitating the death of a cell or a death signal is meant to be construed in its broadest sense meaning that the amino acid sequence plays a role in a pathway leading to cell death. The death signal may also be
- 25 regarded as an apoptotic signal. Although not wishing to limit the present invention to any one theory or mode of action, it is proposed herein that there is a pathway from p75^{NTR} activation to caspase activation and cellular degeneration. It is further proposed that p75^{NTR}-mediated cell death may occur directly or indirectly *via* Bcl-2.

- 30 The present specification refers interchangeably to death signal, death signal region, signalling, inducing or otherwise facilitating the death of a cell and C35.

The nucleic acid molecule of the present invention may encode a non-full length p75^{NTR} molecule although to facilitate cell death, the nucleic acid molecule must encode all or part of the cytoplasmic portion of the p75^{NTR} molecule and a sufficient amount of the membrane domain such that the region referred to herein as the death signal is membrane associated. A "part" of the cytoplasmic domain of p75^{NTR} includes all or a death-inducing functional part of a 35 amino acid region juxtaposed to the membrane domain. Alternatively, the cytoplasmic domain of the p75^{NTR} molecule is in multimeric form or capable of forming multimers. A multimer comprises two or more copies of the molecule such as a dimer, trimer or larger copy molecule.

10

The term "membrane associated" means that the death signal is adjacent, proximal or otherwise juxtaposed to the membrane of a cell expressing the nucleic acid molecule.

The "death signal region" and other related terms are used herein to describe functionally the region of the cytoplasmic portion of p75^{NTR} which is adjacent, proximal or otherwise juxtaposed to a region of p75^{NTR} which associates with the membrane or which cytoplasmic portion is in multimeric form. The death signal region is not the same portion of the molecules as the "death domain" [9] although there may be functional similarities in death signalling.

20

Accordingly, another aspect of the present invention is directed to a nucleic acid molecule comprising a sequence of nucleotides or complementary sequence of nucleotides which encodes a peptide, polypeptide or protein capable of signalling, inducing or otherwise facilitating death of a cell in which it is expressed wherein said peptide, polypeptide or protein comprises a membrane associating portion and/or a multimer-forming portion and a portion which corresponds to all or part of the cytoplasmic region of p75^{NTR} or a functional equivalent, derivative or homologue thereof.

In order to signal, induce or otherwise facilitate death of a cell, the death signal region is preferably adjacent, proximal or otherwise juxtaposed to the cell membrane. However, the present invention extends to multimeric forms and attachments which facilitate same. A

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multimer comprises two or more molecules.

In one embodiment, the membrane portion is derived from p75^{NTR} or a functional equivalent, derivative or homologue thereof. In another embodiment, the membrane domain is from
5 another molecule such as a receptor or other ligand-binding molecule. Examples of receptors according to this aspect of the present invention include cytokine receptors (e.g. the Leukaemia Inhibitory Factor (LIF) receptor, interleukin receptor, and colony-stimulating factor receptors). Examples of ligand-binding molecules include immunoglobulins and T cell receptors.

10

When in multimeric form, the molecule is only optionally associated with the membrane to effect cell death.

The nucleic acid molecule may comprise cDNA or genomic DNA or may comprise
15 ribonucleotides such as mRNA. The nucleic acid molecule may be derived from a cDNA or genomic molecule encoding p75^{NTR} or a derivative or homologue thereof or may be prepared by the stepwise addition of nucleotides in a defined sequence.

The nucleic acid molecule of the present invention may also be considered as corresponding
20 to a "gene".

Reference herein to a "gene" is to be taken in its broadest context and includes:

- (i) a classical genomic gene consisting of transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (i.e.
25 introns, 5'- and 3'- untranslated sequences);
- (ii) mRNA or cDNA corresponding to the coding regions (i.e. exons) optionally comprising 5'- or 3'-untranslated sequences of the gene; or
- (iii) an amplified DNA fragment or other recombinant nucleic acid molecule
30 produced *in vitro* and comprising all or a part of the coding region and/or 5'- or 3'- untranslated sequences of the gene.

The term "gene" is also used to describe synthetic or fusion molecules encoding all or part of a functional product. A functional product is one which comprises a sequence of nucleotides or is complementary to a sequence of nucleotides which encodes a functional death signal from p75^{NTR} or its derivative or homologue.

5

The nucleotide sequence of the present invention may correspond to the cDNA or genomic sequence of a gene encoding p75^{NTR} or a death signal region thereof or may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or additions.

Nucleotide insertional derivatives of the nucleic acid molecule of the present invention

10 include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides. Insertional nucleotide sequence variants are those in which one or more nucleotides are introduced into a predetermined site in the nucleotide sequence although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterised by the removal of one or more nucleotides from the sequence.

15 Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide inserted in its place. Such a substitution may be "silent" in that the substitution does not change the amino acid defined by the codon.

Alternatively, substituents are designed to alter one amino acid for another similar acting amino acid, or amino acid of like charge, polarity, or hydrophobicity.

20

Accordingly, another aspect of the present invention contemplates homologues, analogues and derivatives of a nucleic acid molecule which encodes a peptide, polypeptide or protein which is capable of signalling, inducing or otherwise facilitating death of a cell in which it is expressed wherein said peptide, polypeptide or protein comprises a membrane associating

25 portion and/or multimer-forming portion and a portion which corresponds to all or part of the cytoplasmic region of p75^{NTR} or a functional equivalent, derivative or homologue thereof.

For the present purpose, "homologues" of a nucleic acid molecule as herein defined or of a nucleotide sequence shall be taken to refer to an isolated nucleic acid molecule which is

30 substantially the same as the nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence within said sequence, of

- 10 -

one or more nucleotide substitutions, insertions, deletions, or rearrangements.

"Analogues" of a nucleic acid molecule as herein defined or of a nucleotide sequence set forth herein shall be taken to refer to an isolated nucleic acid molecule which is substantially the same as a nucleic acid molecule of the present invention or its complementary nucleotide sequence, notwithstanding the occurrence of any non-nucleotide constituents not normally present in said isolated nucleic acid molecule, for example carbohydrates, radiochemicals including radionucleotides, reporter molecules such as, but not limited to DIG, alkaline phosphatase or horseradish peroxidase, amongst others.

10

"Derivatives" of a nucleic acid molecule as herein defined or of a nucleotide sequence set forth herein shall be taken to refer to any isolated nucleic acid molecule which contains significant sequence similarity to said sequence or a part thereof. Generally, the nucleotide sequence of the present invention may be subjected to mutagenesis to produce single or multiple nucleotide substitutions, deletions and/or insertions. Nucleotide insertional derivatives of the nucleotide sequence of the present invention include 5' and 3' terminal fusions as well as intra-sequence insertions of single or multiple nucleotides or nucleotide analogues. Insertional nucleotide sequence variants are those in which one or more nucleotides or nucleotide analogues are introduced into a predetermined site in the nucleotide sequence of said sequence, although random insertion is also possible with suitable screening of the resulting product being performed. Deletional variants are characterised by the removal of one or more nucleotides from the nucleotide sequence. Substitutional nucleotide variants are those in which at least one nucleotide in the sequence has been removed and a different nucleotide or nucleotide analogue inserted in its place.

25

In one embodiment, the derivatives encode a peptide, polypeptide or protein which induces cell death. In another embodiment, the derivatives do not induce cell death but antagonise the death signal.

30

The nucleic acid molecule of the present invention may be based on a nucleotide sequence of the gene or cDNA encoding p75^{NTR} from any animal such as from mammals. Preferred mammals include humans, primates, livestock animals (e.g. cows, sheep, horses, pigs, donkeys, goats), laboratory test animals (e.g. rabbits, mice, rats, guinea pigs, hamsters),
5 companion animals (e.g. dogs, cats) and captive wild animals.

A particularly preferred sequence is from human or primate or murine p75^{NTR}.

The present invention is exemplified using a nucleotide sequence from rat p75^{NTR} cDNA.
10 This is done, however, with the understanding that the nucleotide sequence may be from p75^{NTR} genomic or cDNA from any animal.

Accordingly, another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence or a complementary form thereof wherein said nucleotide
15 sequence is capable of hybridising to SEQ ID NO:1 under low stringency conditions at 42 °C.

The nucleotide sequence set forth in SEQ ID NO:1 is the cDNA sequence encoding p75^{NTR}.
The nucleic acid molecule according to this aspect of the present invention does not extend to the full length p75^{NTR} cDNA sequence but comprises a portion which encodes an amino acid
20 sequence which signals, induces or otherwise facilitates cell death when associated with a membrane portion of p75^{NTR} or other molecules.

Accordingly, another aspect of the present invention provides a nucleic acid molecule comprising a nucleotide sequence or complementary nucleotide sequence which is
25 substantially as set forth in SEQ ID NO:7 or is a nucleotide sequence capable of hybridising thereto under low stringency conditions at 42 °C or is a nucleotide sequence having at least 60% identity thereto.

The nucleotide sequence set forth in SEQ ID NO:7 is the death signal defined herein
30 associated with p75^{NTR}. This sequence encodes a 35 amino acid region also referred to herein as "C35".

Reference herein to a low stringency at 42°C includes and encompasses from at least about 1% v/v to at least about 15% v/v formamide and from at least about 1M to at least about 2M salt for hybridisation, and at least about 1M to at least about 2M salt for washing conditions. Alternative stringency conditions may be applied where necessary, such as medium

5 stringency, which includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5M to at least about 0.9M salt for hybridisation, and at least about 0.5M to at least about 0.9M salt for washing conditions, or high stringency, which includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01M to at least about 0.15M salt for

10 hybridisation, and at least about 0.01M to at least about 0.15M salt for washing conditions.

The present invention further contemplates a nucleic acid molecule comprising a nucleotide sequence or a complementary form thereof, which nucleotide sequence encodes an amino acid sequence substantially as set forth in SEQ ID NO:8 or a derivative, homologue or

15 chemical equivalent thereof or an amino acid sequence having at least 60% identity thereto.

The amino acid sequence of SEQ ID NO:8 corresponds to the amino acid sequence of the p75^{NTR} death signal.

20 The term "identity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, the term "similarity" may also be used and includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid

25 level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In a particularly preferred embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity. Any number of programs are available to compare nucleotide and amino acid sequences. Preferred programs have regard to an appropriate alignment. One such program

30 is Gap which considers all possible alignment and gap positions and creates an alignment with the largest number of matched bases and the fewest gaps. Gap uses the alignment method of

Needleman and Wunsch [17]. Gap reads a scoring matrix that contains values for every possible GCG symbol match. GAP is available on ANGIS (Australian National Genomic Information Service) at website <http://mell.angis.org.au..>

- 5 The present invention further comprises a nucleic acid molecule comprising the nucleotide sequence:

$$\{n_1 - - - n_x\}_b \text{ a } \{n'_1 - - - n'_y\}_c \text{ a } \{n''_1 - - - n''_z\}_d$$

wherein

- 10 $\{n_1 - - - n_x\}$ is a sequence of x nucleotides encoding an extracellular portion of a receptor or ligand-binding molecule;

$\{n'_1 - - - n'_y\}$ is a sequence of y nucleotides encoding a transmembrane peptide, polypeptide or protein or a molecule capable of inducing multimerisation;

- 15 $\{n''_1 - - - n''_z\}$ is a sequence of z nucleotides comprising a nucleotide sequence substantially as set forth in SEQ ID NO:7 or a nucleotide sequence encoding an amino acid sequence substantially as set forth in SEQ ID NO:8 or a nucleotide sequence capable of hybridising thereto under low stringency conditions at 42 °C or a nucleotide sequence having at least 60% identity to SEQ ID NO:7;

20

b, c and d may be the same or different and each is 0, 1 or >1;

x, y and z may be the same or different and each is 0, 1 or >1;

25

a is a nucleotide bond;

wherein when c is 1 or >1 and d is 1 or >1 and wherein when the molecule is expressed in a neuronal cell, the expression product signals, induces or otherwise facilitates cell death.

30

Preferably, $\{n_1 - - - n_x\}$ comprises the nucleotide sequence substantially as set forth in SEQ ID NO:3 or is a nucleotide sequence having at least about 60% identity thereto or is capable of hybridising thereto under low stringency conditions at 42 °C.

- 5 Preferably, $\{n'_1 - - - n'_y\}$ comprises the nucleotide sequence substantially as set forth in SEQ ID NO:5 or is a nucleotide sequence having at least about 60% identity thereto or is capable of hybridising thereto under low stringency conditions at 42 °C.

The nucleotide sequences $\{n_1 - - - n_x\}$, $\{n'_1 - - - n'_y\}$ and $\{n''_1 - - - n''_z\}$ may be in any order
10 and in any combination.

For the production of a recombinant peptide, polypeptide or protein comprising the death signal, the nucleic acid molecule of the present invention is placed, in the sense orientation, in operable connection with a suitable promoter sequence and introduced into a suitable
15 expression system, for example a bacterial, yeast, baculovirus, plant, animal or other expression system.

Accordingly, a further aspect of the present invention provides a genetic construct comprising an isolated nucleic acid molecule which comprises a sequence of nucleotides which
20 corresponds or is complementary to a death signal region from p75^{NTR} or a homologue, analogue or derivative thereof.

According to this embodiment, the coding region of the death signal from p75^{NTR} may be placed in operable connection with a promoter sequence such that a gene product is capable
25 of being expressed under the control of said promoter sequence.

Optionally, said genetic construct further comprises a terminator sequence.

In the present context, the term "in operable connection with" is used to indicate that
30 expression of the isolated nucleotide sequence is under the control of the promoter sequence with which it is connected.

The term "terminator" refers to a DNA sequence at the end of a transcriptional unit which signals termination of transcription. Terminators are 3'-non-translated DNA sequences containing a polyadenylation signal, which facilitates the addition of polyadenylate sequences to the 3'-end of a primary transcript. Terminators active in plant cells are known and
5 described in the literature. They may be isolated from bacteria, fungi, viruses, animals and/or plants.

Examples of terminators particularly suitable for use in the genetic constructs of the present invention include the SV40 polyadenylation signal, amongst others.

10

Reference herein to a "promoter" is to be taken in its broadest context and includes the transcriptional regulatory sequences of a classical genomic gene, including the TATA box which is required for accurate transcription initiation in eukaryotic cells, with or without a CCAAT box sequence and additional regulatory elements (i.e. upstream activating sequences,
15 enhancers and silencers). For expression in prokaryotic cells, such as bacteria, the promoter should at least contain the -35 box and -10 box sequences.

A promoter is usually, but not necessarily, positioned upstream or 5', of the nucleotide sequence encoding the death signal of p75^{NTR}, the expression of which it regulates.

20 Furthermore, the regulatory elements comprising a promoter are usually positioned within 2 kb of the start site of transcription of the gene.

In the present context, the term "promoter" is also used to describe a synthetic or fusion molecule, or derivative which confers, activates or enhances expression of an isolated nucleic
25 acid molecule, in a cell, such as a plant, animal, insect, fungal, yeast or bacterial cell.

Preferred promoters may contain additional copies of one or more specific regulatory elements, to further enhance expression of a nucleic acid molecule which expression it regulates and/or to alter the spatial expression and/or temporal expression of same. For example, regulatory elements which confer copper inducibility may be placed adjacent to a
30 heterologous promoter sequence driving expression of a nucleic acid molecule, thereby conferring copper inducibility on the expression of said molecule.

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Placing an isolated nucleic acid molecule under the regulatory control of a promoter sequence means positioning said molecule such that expression is controlled by the promoter sequence. Promoters are generally positioned 5' (upstream) to the genes that they control. In the construction of heterologous promoter/structural gene combinations it is generally preferred
5 to position the promoter at a distance from the gene transcription start site that is approximately the same as the distance between that promoter and the gene it controls in its natural setting, i.e., the gene from which the promoter is derived. As is known in the art, some variation in this distance can be accommodated without loss of promoter function. Similarly, the preferred positioning of a regulatory sequence element with respect to a
10 heterologous gene to be placed under its control is defined by the positioning of the element in its natural setting, i.e., the genes from which it is derived. Again, as is known in the art, some variation in this distance can also occur.

Examples of promoters suitable for use in genetic constructs of the present invention include
15 viral, fungal, bacterial, animal and plant derived promoters capable of functioning in plant, animal, insect, fungal, yeast or bacterial cells. The promoter may regulate the expression of the nucleic acid molecule constitutively, or differentially with respect to the tissue in which expression occurs or, with respect to the developmental stage at which expression occurs, or in response to external stimuli such as physiological stresses, or plant pathogens, or metal
20 ions, amongst others.

Preferably, the promoter is capable of regulating expression of a nucleic acid molecule in a yeast or bacterial cell.

25 Examples of preferred promoters include the bacteriophage T7 promoter, bacteriophage T3 promoter, SP6 promoter, *lac* promoter, *tac* promoter, SV40 early promoter, and the like.

The genetic construct contemplated herein is introduced into a suitable expression system for a time and under conditions sufficient for expression of said death signal from p75^{NTR} to
30 occur.

The genetic construct may also comprising a nucleotide sequence corresponding to all or part of the membrane domain of p75^{NTR} or other membrane molecules.

Accordingly, a further aspect of the invention contemplates a recombinant peptide,
5 polypeptide or protein produced by expressing the isolated nucleic acid molecule herein described in a suitable host cell. The present invention extends also to a synthetic peptide fragment of said recombinant gene product.

The present invention further contemplates an isolated peptide, polypeptide or protein
10 comprising the cytoplasmic region of p75^{NTR} which signals, induces or otherwise facilitates cell death when said peptide, polypeptide or protein is adjacent, proximal or otherwise juxtaposed to a membrane-associating region such as from p75^{NTR} or other membrane molecule and/or is in multimeric form or a derivative, homologue, chemical equivalent or analogue of said peptide, polypeptide or protein. This aspect of the present invention does
15 not extend to the full length p75^{NTR}.

Preferably, the peptide, polypeptide or protein comprises an amino acid sequence substantially as set forth in SEQ ID NO:8 or an amino acid sequence having at least 60% identity thereto or a chemical equivalent, derivative, homologue or analogue of said peptide,
20 polypeptide or protein.

The term "isolated" means that the peptide, polypeptide or protein of the present invention is provided in a form which is distinct from that which occurs in nature, preferably wherein one or more contaminants have been removed. Accordingly, the isolated peptide, polypeptide
25 or protein of the invention may be partially-purified or substantially pure, in which a substantial amount of the contaminants have been removed or in sequencably pure or substantially homogeneous form.

The term "sequencably pure" means that the isolated peptide, polypeptide or protein is
30 provided in a form which is sufficiently purified to facilitate amino acid sequence determination using procedures known to those skilled in the art.

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The term "substantially homogeneous" means that the isolated peptide, polypeptide or protein of the present invention is at least about 95% free of contaminants, more preferably at least about 99% free of contaminants, including 100% purity.

- 5 The present invention extends to a range of derivatives and chemical analogues of the peptide, polypeptide or protein.

Furthermore, the amino acids of a homologous polypeptide may be replaced by other amino acids having similar properties, for example hydrophobicity, hydrophilicity, hydrophobic
10 moment, charge or antigenicity, and so on.

"Analogues" encompass death signal containing peptides, polypeptides or proteins which are at least about 60% identical to the p75^{NTR} death signal sequence (SEQ ID NO:8), notwithstanding the occurrence of any non-naturally occurring amino acid analogues therein.
15 "Analogues" also encompass polypeptide mimotypes.

The term "derivative" in relation to a peptide, polypeptide or protein shall be taken to refer hereinafter to mutants, parts or fragments derived from the functional p75^{NTR} molecule or death signal region thereof or derivatives thereof which may or may not possess the death
20 signal activity of the functional p75^{NTR}. Derivatives include modified peptides in which ligands are attached to one or more of the amino acid residues contained therein, such as carbohydrates, enzymes, proteins, polypeptides or reporter molecules such as radionuclides or fluorescent compounds. Glycosylated, fluorescent, acylated or alkylated forms of the subject peptides are particularly contemplated by the present invention. Additionally,
25 derivatives of the peptide, polypeptide or protein described herein comprise fragments or parts of an amino acid sequence disclosed herein are within the scope of the invention, as are homopolymers or heteropolymers comprising two or more copies of the subject polypeptides. Procedures for derivatizing peptides are well-known in the art.

A homologue, analogue or derivative of SEQ ID NO:2 or SEQ ID NO:8 may comprise an amino acid substitution or said SEQ ID NO: 2 or 8 may encompass amino acid alterations in which an amino acid is replaced with a different naturally-occurring or a non-conventional amino acid residue. Such substitutions may be classified as "conservative", in which case an amino acid residue contained in a phospholipase inhibitory protein is replaced with another naturally-occurring amino acid of similar character, for example Gly↔Ala, Val↔Ile↔Leu, Asp↔Glu, Lys↔Arg, Asn↔Gln or Phe↔Trp↔Tyr.

Substitutions encompassed by the present invention may also be "non-conservative", in which an amino acid residue which is present in a phospholipase inhibitory protein is substituted with an amino acid having different properties, such as a naturally-occurring amino acid from a different group (eg. substituted a charged or hydrophobic amino acid with alanine), or alternatively, in which a naturally-occurring amino acid is substituted with a non-conventional amino acid.

Amino acid substitutions are typically of single residues, but may be of multiple residues, either clustered or dispersed.

Naturally-occurring amino acids include those listed in Table 1. Non-conventional amino acids encompassed by the invention include, but are not limited to those listed in Table 2.

Amino acid deletions will usually be of the order of about 1-10 amino acid residues, while insertions may be of any length. Deletions and insertions may be made to the N-terminus, the C-terminus or be internal deletions or insertions. Generally, insertions within the amino acid sequence will be smaller than amino-or carboxyl-terminal fusions and of the order of 1-4 amino acid residues.

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TABLE 1

	Amino Acid	Three-letter Abbreviation	One-letter Symbol
5			
	Alanine	Ala	A
	Arginine	Arg	R
	Asparagine	Asn	N
10	Aspartic acid	Asp	D
	Cysteine	Cys	C
	Glutamine	Gln	Q
	Glutamic acid	Glu	E
	Glycine	Gly	G
15	Histidine	His	H
	Isoleucine	Ile	I
	Leucine	Leu	L
	Lysine	Lys	K
	Methionine	Met	M
20	Phenylalanine	Phe	F
	Proline	Pro	P
	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
25	Tyrosine	Tyr	Y
	Valine	Val	V
	Any amino acid as above	Xaa	X

TABLE 2

	Non-conventional amino acid	Code	Non-conventional amino acid	Code
5				
	α -aminobutyric acid	Abu	L-N-methylalanine	Nmala
	α -amino- α -methylbutyrate	Mgab	L-N-methylarginine	Nmarg
	aminocyclopropane-	Cpro	L-N-methylasparagine	Nmasn
	carboxylate		L-N-methylaspartic acid	Nmasp
10	aminoisobutyric acid	Aib	L-N-methylcysteine	Nmcys
	aminonorbornyl-	Norb	L-N-methylglutamine	Nmgln
	carboxylate		L-N-methylglutamic acid	Nmglu
	cyclohexylalanine	Chexa	L-N-methylhistidine	Nmhis
	cyclopentylalanine	Cpen	L-N-methylisoleucine	Nmile
15	D-alanine	Dal	L-N-methylleucine	Nmleu
	D-arginine	Darg	L-N-methyllysine	Nmlys
	D-aspartic acid	Das	L-N-methylmethionine	Nmmet
	D-cysteine	Dcys	L-N-methylnorleucine	Nmnle
	D-glutamine	Dgln	L-N-methylnorvaline	Nmnva
20	D-glutamic acid	Dglu	L-N-methylornithine	Nmorn
	D-histidine	Dhis	L-N-methylphenylalanine	Nmphe
	D-isoleucine	Dile	L-N-methylproline	Nmpro
	D-leucine	Dleu	L-N-methylserine	Nmser
	D-lysine	Dlys	L-N-methylthreonine	Nmthr
25	D-methionine	Dmet	L-N-methyltryptophan	Nmtrp
	D-ornithine	Dorn	L-N-methyltyrosine	Nmtyr
	D-phenylalanine	Dphe	L-N-methylvaline	Nmval
	D-proline	Dpro	L-N-methylethylglycine	Nmetg
	D-serine	Dser	L-N-methyl-t-butylglycine	Nmtbug
30	D-threonine	Dthr	L-norleucine	Nle
	D-tryptophan	Dtrp	L-norvaline	Nva

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	D-tyrosine	Dtyr	α -methyl-aminoisobutyrate	Maib
	D-valine	Dval	α -methyl- γ -aminobutyrate	Mgab
	D- α -methylalanine	Dmala	α -methylcyclohexylalanine	Mchexa
	D- α -methylarginine	Dmarg	α -methylcyclopentylalanine	Mcpen
5	D- α -methylasparagine	Dmasn	α -methyl- α -naphthylalanine	Manap
	D- α -methylaspartate	Dmasp	α -methylpenicillamine	Mpen
	D- α -methylcysteine	Dmcys	N-(4-aminobutyl)glycine	Nglu
	D- α -methylglutamine	Dmgln	N-(2-aminoethyl)glycine	Naeg
	D- α -methylhistidine	Dmhis	N-(3-aminopropyl)glycine	Norn
10	D- α -methylisoleucine	Dmile	N-amino- α -methylbutyrate	Nmaabu
	D- α -methylleucine	Dmleu	α -naphthylalanine	Anap
	D- α -methyllysine	Dmlys	N-benzylglycine	Nphe
	D- α -methylmethionine	Dmmet	N-(2-carbamylethyl)glycine	Ngln
	D- α -methylornithine	Dmorn	N-(carbamylmethyl)glycine	Nasn
15	D- α -methylphenylalanine	Dmphe	N-(2-carboxyethyl)glycine	Nglu
	D- α -methylproline	Dmpro	N-(carboxymethyl)glycine	Nasp
	D- α -methylserine	Dmser	N-cyclobutylglycine	Ncbut
	D- α -methylthreonine	Dmthr	N-cycloheptylglycine	Nchep
	D- α -methyltryptophan	Dmtrp	N-cyclohexylglycine	Nchex
20	D- α -methyltyrosine	Dmty	N-cyclodecylglycine	Ncdcc
	D- α -methylvaline	Dmval	N-cyclododecylglycine	Ncdod
	D-N-methylalanine	Dnmala	N-cyclooctylglycine	Ncoct
	D-N-methylarginine	Dnmarg	N-cyclopropylglycine	Ncpro
	D-N-methylasparagine	Dnmasn	N-cycloundecylglycine	Ncund
25	D-N-methylaspartate	Dnmasp	N-(2,2-diphenylethyl) glycine	Nbhm
	D-N-methylcysteine	Dnmcys	N-(3,3-diphenylpropyl) glycine	Nbhe

	D-N-methylglutamine	Dnmglu	N-(3-guanidinopropyl) glycine	Narg
	D-N-methylglutamate	Dnmglu	N-(1-hydroxyethyl)glycine	Nthr
	D-N-methylhistidine	Dnmhis	N-(hydroxyethyl)glycine	Nser
5	D-N-methylisoleucine	Dnmile	N-(imidazolylethyl) glycine	Nhis
	D-N-methylleucine	Dnmleu	N-(3-indolylyethyl) glycine	Nhtrp
	D-N-methyllysine	Dnmlys	N-methyl- γ -aminobutyrate	Nmgabu
10	N-methylcyclohexylalanine	Nmchexa	D-N-methylmethionine	Dnmmt
	D-N-methylornithine	Dnmorn	N-methylcyclopentylalanine	Nmcpn
	N-methylglycine	Nala	D-N-methylphenylalanine	Dnmphe
	N-methylaminoisobutyrate	Nmaib	D-N-methylproline	Dnmpro
	N-(1-methylpropyl)glycine	Nile	D-N-methylserine	Dnmser
15	N-(2-methylpropyl)glycine	Nleu	D-N-methylthreonine	Dnmthr
	D-N-methyltryptophan	Dnmtrp	N-(1-methylethyl)glycine	Nval
	D-N-methyltyrosine	Dnmtyr	N-methyl- α -naphthylalanine	Nmanap
	D-N-methylvaline	Dnmval	N-methylpenicillamine	Nmpen
	γ -aminobutyric acid	Gabu	N-(<i>p</i> -hydroxyphenyl)glycine	Nhtyr
20	L- <i>t</i> -butylglycine	Tbug	N-(thiomethyl)glycine	Ncys
	L-ethylglycine	Etg	penicillamine	Pen
	L-homophenylalanine	Hphe	L- α -methylalanine	Mala
	L- α -methylarginine	Marg	L- α -methylasparagine	Masn
	L- α -methylaspartate	Masp	L- α -methyl- <i>t</i> -butylglycine	Mtbug
25	L- α -methylcysteine	Mcys	L-methylethylglycine	Metg
	L- α -methylglutamine	Mgln	L- α -methylglutamate	Mglu
	L- α -methylhistidine	Mhis	L- α -methylhomo phenylalanine	Mhphe
	L- α -methylisoleucine	Mile	N-(2-methylthioethyl) glycine	Nmet
30	L- α -methylleucine	Mleu	L- α -methyllysine	Mlys

L- α -methylmethionine	Mmet	L- α -methylnorleucine	Mnle
L- α -methylnorvaline	Mnva	L- α -methylornithine	Morn
L- α -methylphenylalanine	Mphe	L- α -methylproline	Mpro
L- α -methylserine	Mser	L- α -methylthreonine	Mthr
5 L- α -methyltryptophan	Mtrp	L- α -methyltyrosine	Mtyr
L- α -methylvaline	Mval	L-N-methylhomo	
		phenylalanine	Nmhpe
N-(N-(2,2-diphenylethyl)		N-(N-(3,3-diphenylpropyl)	
carbamylmethyl)glycine	Nnbhm	carbamylmethyl)glycine	Nnbhe
10 1-carboxy-1-(2,2-diphenyl-			
ethylamino)cyclopropane	Nmbc		

The present invention provides for a method of treatment or prophylaxis of disease conditions
 15 associated with neuronal death or where cell death is to be promoted such as in treating or
 preventing cancer growth and/or development.

In one embodiment, it has been determined in accordance with the present invention that
 expression of a nucleic acid molecule encoding only death signal and not adjacent, proximal
 20 or juxtaposed to a membrane-associating sequence results in antagonising of the death signal.

According to this embodiment, the present invention contemplates a method for inhibiting,
 reducing or otherwise antagonising a p75^{NTR}-mediated death signal in a neuronal cell, said
 method comprising introducing a nucleic acid molecule capable of being expressed to an
 25 expression product which corresponds to a non-membrane associated form of the p75^{NTR}
 death signal region or a derivative, functional equivalent or homologue thereof.

This aspect of the present invention is useful for the treatment of a range of
 neurodegenerative diseases such as cerebral palsy, trauma induced paralysis, vascular
 30 ischaemia associated with stroke, neuronal tumours, motoneurone disease, Parkinson's
 disease, Huntington's disease, Alzheimer's disease, multiple sclerosis and peripheral



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neuropathies associated with diabetes, heavy metal or alcohol toxicity, renal failure and/or infectious diseases such as herpes, rubella, measles, chicken pox, HIV and/or HTLV-1. This aspect is also useful for treating neurons damaged by trauma or disease.

- 5 Alternatively, the method is aimed at targetting certain cells such as cancer cells wherein expression is required of a death signal from p75^{NTR} or a derivative, functional equivalent or homologue thereof adjacent, proximal or otherwise juxtaposed to a membrane-associating portion of p75^{NTR} or other membrane molecules or is in multimeric form. The nucleic acid molecule may require modification to ensure appropriate targetting to the cell or the nucleic
- 10 acid molecule may be injected directly into cancerous tissue.

- Another aspect of the present invention provides a biological composition comprising a genetic molecule capable of being expressed into a p75^{NTR} death signal antagonist or a p75^{NTR} death signal. The biological composition further comprises one or more pharmaceutically
- 15 acceptable carriers and/or diluents. The nucleic acid molecules according to this aspect of the present invention may be naked nucleic acid molecules or contained or associated with a viral vector or other suitable delivery mechanism.

- The present invention is also useful as a culture agent such as preventing or reducing the
- 20 death of cells *in vitro*. The present invention is particularly useful *in vitro* when used in combination with LIF. Even more particularly, the present invention is useful for culturing recombinant cell lines.

- The present invention also provides for the use of the death signal of p75^{NTR} in the
- 25 manufacture of a medicament for the treatment of neurodegenerative diseases in animals. Preferred animals include humans, primates, livestock animals, laboratory test animals, companion animals and captive wild animals.



The present invention is further described by the following non-limiting Examples.

EXAMPLE 1

The aim of this example was to determine the protein domains on p75^{NTR} responsible for
5 death signalling.

In order to investigate how p75^{NTR} signals neuronal death, the inventors devised a robust *in vitro* assay for p75^{NTR} induced death. Plasmid expression constructs were microinjected into individual neurons in the presence of the growth factor LIF, and the survival of the neurons
10 expressing the different plasmids was determined. A series of plasmid constructs which encode incomplete p75^{NTR} proteins were made (see Figure 1) and the ability of each protein to signal death when over expressed was assessed.

The p75^{NTR} protein is a transmembrane protein comprised of a large extracellular domain
15 with four cysteine rich motifs responsible for interacting with soluble growth factors, and a short cytoplasmic, intracellular tail. The cytoplasmic domain does not contain a kinase domain but contains a domain with significant homology to a motif known as a "death domain", found in apoptosis-inducing Tumour Necrosis Factor Receptors (TNFR) and TNFR-associating death-effector proteins [9].

20 Using expression plasmids of p75^{NTR} proteins deleted for either the entire cytoplasmic domain (p75nc) or a significant portion of the cytoplasmic domain including the entire death domain (p75tm), the inventors found that the cytoplasmic domain is responsible for death signalling. Surprisingly, the intracellular 35 amino acid domain juxtaposed to the membrane, and not the
25 death domain, is responsible for death signalling (Figure 2). This region of the p75^{NTR} protein shows no homology to other death inducing proteins or to known functional motifs.

To further investigate the domain required for death signalling the inventors made constructs expressing p75^{NTR} proteins deleted for the extracellular domain or the extracellular and
30 transmembrane domains. Proteins without extracellular domains retain the signal peptide which is responsible for correctly transporting the protein into the cell membrane. Proteins



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without transmembrane domains are expressed free in the cytoplasm of the cell and are epitope tagged with a FLAG motif for detection.

5 The inventors found that the extracellular domain of p75^{NTR} had a significant inhibitory effect of the ability of the cytoplasmic domain to signal cell death. Furthermore, the membrane linked 35 amino acid cytoplasmic domain (c35) was a potent stimulant of neuronal death with over 90% of cells injected with the plasmid dead after 16 hours (Figure 3). However, if the cytoplasmic 35 amino acid domain is not associated with the membrane, the ability of the domain to induce death is removed (Figure 4).

10

These results indicate that the domain responsible for death induction is within the first 35 amino acids of the cytoplasmic tail but that the transmembrane domain, or at least association with the membrane, is required for death-signal activation. This may be related to the ability of the transmembrane protein to more efficiently form death-signal inducing multimers, or
15 that the position of the p75^{NTR} protein in relation to other membrane-bound accessory molecules might be important in initiating death signalling.

The inventors hypothesised that the free cytoplasmic expressed 35 amino acid domain might be able to interfere with death signalling from full length p75^{NTR} proteins by a dominant-
20 negative mechanism, and attempted to inhibit the death by co-expressing the proteins. Given the results presented below regarding the ability of overexpression of Bcl-2 to enhance p75^{NTR} killing, this paradigm was used to test the ability of the c35 protein to inhibit death signalling. The inventors found that indeed the expression of the c35 protein was able to inhibit this killing (Figure 5). This further indicates that p75^{NTR} signals killing *via* interaction
25 of an accessory molecule to a motif within the first 35 amino acids of the cytoplasmic domain.

EXAMPLE 2

The aim of this example is to determine the minimum number of amino acid residues on c35 require to mediate death signalling.

- 5 A series of deletion and truncation mutants in the c35 region are produced and tested for the ability to induce death signalling.

The deletion mutants from the membrane distal end are as follows:

- 10 KRWNSCKQNKQGANSRPVNQTPPPEGEKLHSDSG;
 KRWNSCKQNKQGANSRPVNQTPPPEGEKLHSDS;
 KRWNSCKQNKQGANSRPVNQTPPPEGEKLHSD;
 KRWNSCKQNKQGANSRPVNQTPPPEGEKLHS;
 KRWNSCKQNKQGANSRPVNQTPPPEGEKLH;
 15 KRWNSCKQNKQGANSRPVNQTPPPEGEKL;
 KRWNSCKQNKQGANSRPVNQTPPPEGEK;
 KRWNSCKQNKQGANSRPVNQTPPPEGE;
 KRWNSCKQNKQGANSRPVNQTPPPEG;
 KRWNSCKQNKQGANSRPVNQTPPPE;
 20 KRWNSCKQNKQGANSRPVNQTPPP;
 KRWNSCKQNKQGANSRPVNQTPP;
 KRWNSCKQNKQGANSRPVNQTP;
 KRWNSCKQNKQGANSRPVNQT;
 KRWNSCKQNKQGANSRPVNQ;
 25 KRWNSCKQNKQGANSRPVN;
 KRWNSCKQNKQGANSRPV;
 KRWNSCKQNKQGANSRP;
 KRWNSCKQNKQGANSR;
 KRWNSCKQNKQGANS;
 30 KRWNSCKQNKQGAN;
 KRWNSCKQNKQGA;



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KRWNSCKQNKQG;
 KRWNSCKQNKQ;
 KRWNSCKQNK;
 KRWNSCKQN;
 5 KRWNSCKQ;
 KRWNSCK;
 KRWNSC;
 KRWNS;
 KRWN;
 10 KRW;
 KR; and
 K.

The deletion mutants from the membrane proximal end are as follows:

15
 RWNSCKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
 WNSCKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
 NSCKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
 SCKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
 20 CKQNKQGANSRPVNQTPPPEGEKLHSDSGI;
 KQNKQGANSRPVNQTPPPEGEKLHSDSGI;
 QNKQGANSRPVNQTPPPEGEKLHSDSGI;
 NKQGANSRPVNQTPPPEGEKLHSDSGI;
 KQGANSRPVNQTPPPEGEKLHSDSGI;
 25 QGANSRPVNQTPPPEGEKLHSDSGI;
 GANSRPVNQTPPPEGEKLHSDSGI;
 ANSRPVNQTPPPEGEKLHSDSGI;
 NSRPVNQTPPPEGEKLHSDSGI;
 SRPVNQTPPPEGEKLHSDSGI;
 30 RPNVNQTPPPEGEKLHSDSGI;
 PVNQTPPPEGEKLHSDSGI;



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VNQTTPPEGEKLHSDSGI;

NQTTPPEGEKLHSDSGI;

QTTPPEGEKLHSDSGI;

TPPPEGEKLHSDSGI;

5 PPPEGEKLHSDSGI;

PPEGEKLHSDSGI;

PEGEKLHSDSGI;

EGEKLHSDSGI;

GEKLHSDSGI;

10 EKLHSDSGI;

KLHSDSGI;

LHSDSGI;

HSDSGI;

SDSGI;

15 DSGI;

SGI;

GI; and

I.

20

EXAMPLE 3**ROLE OF BCL-2 IN PROMOTING p75^{NTR} MEDIATED DEATH SIGNALLING**

As the inventors had shown that the death of dorsal root ganglia (DRG) sensory neurons *in vitro* and *in vivo*, was, at least in part, mediated by p75^{NTR}, p75^{NTR} was over-expressed in these cells by microinjecting rat p75^{NTR} cDNA expressing plasmid into the nucleus of mouse sensory neurons. These were cultured in the presence of the LIF to prevent neuronal death not linked to p75^{NTR} mechanisms. It was found that the expression of the rat p75^{NTR} could be detected by surface immunofluorescence within 24 hours of injection. The injected neurons were observed over a 48 hour period and the viability was assessed. It was found that within the first 16 hours, a significantly higher number of p75^{NTR} plasmid injected neurons had died compared to neurons injected with control plasmids β -galactosidase, or a truncated p75^{NTR} protein lacking the entire cytoplasmic domain (p75^{NTR}nc). It was found that p75^{NTR}-mediated neuronal death occurred later in the experiment similar to Fas/TNF-induced rapid cell death. Since both full-length p75^{NTR} and p75^{NTR}nc protein showed a similar level of expression after injection, this indicates that the cytoplasmic domain of p75^{NTR} is required for death signalling. This was expected since the cytoplasmic tail contains a sequence with homology to the Fas/TNFR "death domain" [9].

The inventors next examined whether deletion of the "death domain" also abolished the ability of p75^{NTR} to kill. It was found that the neuronal death observed after expression of p75^{NTR} with a truncated cytoplasmic tail (p75^{NTR}tr) was equivalent to the full-length p75^{NTR} protein. This demonstrated that the "death domain" was not required for p75^{NTR} killing and, since the p75^{NTR} death domain has recently been shown to have a different tertiary structure to TNFR family death domain and does not self-associate *in vitro*, it suggests that the p75^{NTR} "death domain" may not normally function to induce death. Together, these results predict that an alternative pathway involving proteins other than "death domain" adapter proteins, such as TRADD and FADD, is responsible for p75^{NTR}-mediated killing.

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The Bcl-2 family of proteins is involved in mediating apoptotic signalling pathways, and can homodimerise or heterodimerise with other family members. Bcl-2 and Bcl-xL are well characterised inhibitors of stress-induced apoptosis, JNK activation and neuronal death due to growth-factor limitation. However, both are poor inhibitors of Fas and TNFR mediated
5 opoptosis. As it had been shown previously that high levels of Bcl-2 or Bcl-xL blocked neuronal cell death in a variety of models, the inventors examined whether over-expression of these proteins could block the death induced by p75^{NTR}.

The inventors found that over-expression of Bcl-xL protected neurons against p75^{NTR}-
10 induced death, supporting the hypothesis that p75^{NTR} signals through an alternative pathway to TNFR-induced apoptosis. In contrast, while Bcl-2 over-expression alone had no effect on cell survival in the presence of LIF, Bcl-2 in combination with p75^{NTR} over-expression, surprisingly, induced a significant increase in neuronal death above that seen with p75^{NTR} over-expression alone. Bcl-2 in combination with p75^{NTR}nc did not cause significant cell
15 death and furthermore, the cell death observed with p75^{NTR} and Bcl-2 over-expression was totally ablated if the cells were cultured in NGF. Bcl-2 was able to protect against neuronal death induced by NGF withdrawal, but not withdrawal of LIF. Thus, at the same expression levels in the same neuronal population, Bcl-2 was able to prevent or enhance neuronal cell death depending on the nature of the death signal.

20

These results are surprising since Bcl-2 has previously been shown to have similar actions to Bcl-xL in almost all cell-death systems.

To determine whether the paradoxical effect of Bcl-2 on p75^{NTR}-induced killing was related
25 to its kown anti-apoptotic activity, Bcl-2 proteins with inactivating point mutation, G145E, in the "Bcl-2 Homology" BH1 domain and W188A in the BH2 domain were utilised. Like wildtype Bcl-2, expression of either Bcl-2 mutant alone did not effect neuronal survival. In combination with p75^{NTR} expression, the enhanced killing effect seen with Bcl-2 co-expression was abrogated by the G145E mutation, even though the proteins were expressed
30 to comparable levels. Thus, an intact BH1 homology region is required for the death promoting activity of Bcl-2.

Mutation of the equivalent G138 residue in Bcl-xL results in a conformational change between α -helices 4 and 5, disrupting access to the hydrophobic cleft formed by BH1, BH2 and BH3 domains. Therefore, the molecular mechanism by which Bcl-2 participates in the p75^{NTR} killing pathway may be dependent on interactions either directly with the BH domains or with the hydrophobic cleft, as indicated with experiments using the W188A mutation. Co-expression of p75^{NTR} with the Bcl-2 W188A protein not only abrogated the increased p75^{NTR} killing but, more importantly, protected neurons from any p75^{NTR}-induced death, reminiscent of that seen with Bcl-xL. These experiments suggest that the conformation of the Bcl-2 protein is integral to the opposing functions observed herein.

10

The inventors had observed that DRG neurons isolated from newborn mice depleted for p75^{NTR} were less susceptible to NGF withdrawal, as is the case with sympathetic neurons, when compared to neurons from wildtype mice. This is indicative of absent or delayed naturally occurring cell death observed in these mice. The inventors attempted to induce cell death in p75^{NTR} "knock out" DRG neurons by re-introducing p75^{NTR} expression.

15

Surprisingly, apoptosis was not induced by re-expression into "knock out" DRG neurons, the inventors found that neuronal death was significantly increased under these conditions. This implicated an absolute requirement for Bcl-2 in mediating p75^{NTR} killing.

20 The inventors tested, therefore, whether high endogenous Bcl-2 levels might be necessary for successful p75^{NTR}-mediated killing in normal neurons by assaying p75^{NTR} killing in Bcl-2 depleted cells. Endogenous Bcl-2 was down regulated by antisense as previously described. When the Bcl-2 antisense plasmid was injected at the same time as p75^{NTR} plasmids no diminishment in the death signal was seen. If, however, the Bcl-2 antisense was microinjected first (to give time to reduced Bcl-2 production and deplete endogenous Bcl-2; and then a day later the p75^{NTR} or p75^{NTR}nc constructs were microinjected, there was no difference in survival between p75^{NTR} and p75^{NTR}nc expressing neurons, strongly suggesting that endogenous Bcl-2 is required for p75^{NTR} killing effects. To confirm this observation, the inventors isolated neurons from newborn Bcl-2 "knock out" mice (an heterozygous line of mice containing a disrupted Bcl-2 gene) and their wild-type litter mates and compared the effect of p75^{NTR} over-expression with control plasmid p75^{NTR}. It was found that the neurons

30

isolated from Bcl-2 deficient mice were significantly protected from p75^{NTR} killing, showing a 56.9% (n=3) reduction in death compared wildtype neurons, supporting the hypothesis that endogenous Bcl-2 is required for p75^{NTR} killing.

- 5 Bcl-2 has previously been observed to increase cell death when highly expressed both *in vitro* and *in vivo* when expressed at high levels as a transgene, causing increased apoptosis in the brain under a neuron specific promoter, or in photoreceptor cells when expressed specifically under a rhodopsin promoter. Thus, it is possible that the high level of Bcl-2 is able to "prime" the death pathway such that an apoptotic stimulus *via* p75^{NTR} results in rapid cell
10 death.

Bcl-2 and Bcl-xL when cleaved by caspases have also been shown to be capable of promoting apoptosis *in vitro*, with cells expressing non-cleavable mutant Bcl-2 and Bcl-xL proteins showing increased viability compared to cells expressing wildtype proteins. Cleavage of Bcl-
15 2 is possible in this system, however, the Bcl-2 mutations which results in loss of death promoting activity, would not prevent cleavage of Bcl-2, indicating that cleavage of Bcl-2 would only be part of the mechanism by which Bcl-2 promotes killing. In addition, if cleavage was the dominant mechanism, Bcl-xL might be expected to act as a death signalling protein in this system.

20

To investigate whether the p75^{NTR}-Bcl-2 death-signalling cascade was dependent on caspase activation, inhibitors of caspases were employed. In the presence of z-VAD, a nonspecific caspase peptide inhibitor, or after co-expression of modified crmA plasmids, designed to inhibit Group II caspases such as caspases 2 and 3, p75^{NTR}-mediated death was significantly
25 reduced. Similarly, the modified crmA was able to block the killing induced by co-expression of p75^{NTR} and Bcl-2. This indicates that p75^{NTR} induced apoptosis is a caspase dependent pathway and that the mechanism by which Bcl-2 assists killing is through the same pathway.

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Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this
5 specification, individually or collectively, and any and all combinations of any two or more of said steps or features.



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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH

(ii) TITLE OF INVENTION: A METHOD OF MODULATING CELL SURVIVAL AND REAGENTS USEFUL FOR SAME

(iii) NUMBER OF SEQUENCES: 10

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: DAVIES COLLISON CAVE
(B) STREET: 1 LITTLE COLLINS STREET
(C) CITY: MELBOURNE
(D) STATE: VIC
(E) COUNTRY: AUSTRALIA
(F) ZIP: 3000

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: AU PROVISIONAL
(B) FILING DATE: 06-OCT-1998

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: HUGHES, E JOHN L
(C) REFERENCE/DOCKET NUMBER: EJH/EK

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (61) 3 9254 2777
(B) TELEFAX: (61) 3 9254 2770

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3260 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..3260

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ACA	GCT	CCG	GCG	GGC	AGC	AGG	CGC	TGG	AGC	GCA	TCG	CAG	TTC	AGC	TCA	48
Thr	Ala	Pro	Ala	Gly	Ser	Arg	Arg	Trp	Ser	Ala	Ser	Gln	Phe	Ser	Ser	
1				5					10					15		
GCG	CAG	CAC	CAT	CGG	TCT	GCG	GAG	CGG	ACT	GAG	CTA	GAA	GCG	GAG	CGC	96
Ala	Gln	His	His	Arg	Ser	Ala	Glu	Arg	Thr	Glu	Leu	Glu	Ala	Glu	Arg	
			20					25					30			
TGA	CGC	CGG	AGG	CGT	GCA	ATG	AGG	AGG	GCA	GGT	GCT	GCC	TGC	AGC	GCC	144
*	Arg	Arg	Arg	Arg	Ala	Met	Arg	Arg	Ala	Gly	Ala	Ala	Cys	Ser	Ala	
			35				40					45				
ATG	GAC	CGG	CTG	CGC	CTG	CTG	CTG	CTG	CTG	ATT	CTA	GGG	GTG	TCC	TCT	192
Met	Asp	Arg	Leu	Arg	Leu	Leu	Leu	Leu	Leu	Ile	Leu	Gly	Val	Ser	Ser	
	50					55					60					
GGA	GGT	GCC	AAG	GAG	ACA	TGT	TCC	ACA	GGC	CTG	TAC	ACC	CAC	AGC	GGA	240
Gly	Gly	Ala	Lys	Glu	Thr	Cys	Ser	Thr	Gly	Leu	Tyr	Thr	His	Ser	Gly	
65					70				75						80	
GAG	TGC	TGC	AAA	GCC	TGC	AAC	TTG	GGC	GAA	GGC	GTG	GCC	CAG	CCC	TGC	288
Glu	Cys	Cys	Lys	Ala	Cys	Asn	Leu	Gly	Glu	Gly	Val	Ala	Gln	Pro	Cys	
				85					90					95		
GGA	GCC	AAC	CAG	ACC	GTG	TGT	GAA	CCC	TGC	CTG	GAC	AAT	GTT	ACA	TTC	336
Gly	Ala	Asn	Gln	Thr	Val	Cys	Glu	Pro	Cys	Leu	Asp	Asn	Val	Thr	Phe	
			100					105					110			
TCC	GAT	GTG	GTG	AGC	GCC	ACT	GAG	CCG	TGC	AAG	CCG	TGC	ACC	GAG	TGC	384
Ser	Asp	Val	Val	Ser	Ala	Thr	Glu	Pro	Cys	Lys	Pro	Cys	Thr	Glu	Cys	
		115					120					125				
CTG	GGC	CTG	CAG	AGC	ATG	TCC	GCT	CCC	TGT	GTG	GAG	GCA	GAC	GAT	GCA	432
Leu	Gly	Leu	Gln	Ser	Met	Ser	Ala	Pro	Cys	Val	Glu	Ala	Asp	Asp	Ala	
	130					135					140					
GTG	TGC	AGA	TGT	GCC	TAT	GGC	TAC	TAC	CAG	GAC	GAG	GAG	ACT	GGC	CAC	480
Val	Cys	Arg	Cys	Ala	Tyr	Gly	Tyr	Tyr	Gln	Asp	Glu	Glu	Thr	Gly	His	
145					150				155						160	
TGT	GAG	GCT	TGC	AGC	GTG	TGC	GAG	GTG	GGC	TCG	GGA	CTC	GTG	TTC	TCC	528
Cys	Glu	Ala	Cys		Ser	Val	Cys	Glu	Val	Ser	Gly	Leu	Val	Phe	Ser	
				165					170					175		
TGC	CAG	GAC	AAA	CAG	AAC	ACA	GTG	TGT	GAA	GAG	TGC	CCA	GAG	GGC	ACA	576
Cys	Gln	Asp	Lys	Gln	Asn	Thr	Val	Cys	Glu	Glu	Cys	Pro	Glu	Gly	Thr	
			180					185					190			



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TAC Tyr	TCA Ser	GAC Asp 195	GAA Glu	GCC Ala	AAC Asn	CAC His	GTG Val 200	GAC Asp	CCG Pro	TGC Cys	CTA Leu	CCC Pro 205	TGC Cys	ACG Thr	GTG Val	624
TGC Cys	GAG Glu 210	GAC Asp	ACT Thr	GAG Glu	CGC Arg	CAG Gln 215	TTA Leu	CGC Arg	GAG Glu	TGC Cys	ACG Thr 220	CCC Pro	TGG Trp	GCT Ala	GAT Asp	672
GCT Ala 225	GAA Glu	TGC Cys	GAA Glu	GAG Glu	ATC Ile 230	CCT Pro	GGT Gly	CGA Arg	TGG Trp	ATC Ile 235	CCA Pro	AGG Arg	TCT Ser	ACG Thr	CCC Pro 240	720
CCG Pro	GAG Glu	GGC Gly	TCC Ser	GAC Asp 245	AGC Ser	ACA Thr	GCG Ala	CCC Pro	AGC Ser 250	ACC Thr	CAG Gln	GAG Glu	CCT Pro	GAG Glu 255	GTT Val	768
CCT Pro	CCA Pro	GAG Glu	CAA Gln 260	GAC Asp	CTT Leu	GTA Val	CCC Pro	AGT Ser 265	ACA Thr	GTG Val	GCG Ala	GAT Asp	ATG Met 270	GTG Val	ACC Thr	816
ACT Thr	GTG Val	ATG Met 275	GGC Gly	AGC Ser	TCC Ser	CAG Gln	CCT Pro 280	GTA Val	GTG Val	ACC Thr	CGC Arg	GGC Gly 285	ACC Thr	ACC Thr	GAC Asp	864
AAC Asn 290	CTC Leu	ATT Ile	CCT Pro	GTC Val	TAT Tyr	TGC Cys 295	TCC Ser	ATC Ile	TTG Leu	GCT Ala	GCT Ala 300	GTG Val	GTC Val	GTG Val	GGC Gly	912
CTT Leu 305	GTG Val	GCC Ala	TAT Tyr	ATT Ile	GCT Ala 310	TTC Phe	AAG Lys	AGG Arg	TGG Trp	AAC Asn 315	AGC Ser	TGC Cys	AAA Lys	CAA Gln	AAT Asn 320	960
AAA Lys	CAA Gln	GGC Gly	GCC Ala	AAC Asn 325	AGC Ser	CGC Arg	CCC Pro	GTG Val	AAC Asn 330	CAG Gln	ACG Thr	CCC Pro	CCA Pro	CCG Pro 335	GAG Glu	1008
GGA Gly	GAG Glu	AAA Lys	CTG Leu 340	CAC His	AGC Ser	GAC Asp	AGT Ser 345	GGC Gly	ATC Ile	TCT Ser	GTG Val	GAC Asp 350	AGC Ser	CAG Gln	AGC Ser	1056
CTG Leu	CAC His	GAC Asp 355	CAG Gln	CAG Gln	ACC Thr	CAT His	ACG Thr 360	CAG Gln	ACT Thr	GCC Ala	TCA Ser	GGC Gly 365	CAG Gln	GCC Ala	CTC Leu	1104
AAG Lys 370	GGT Gly	GAT Asp	GGC Gly	AAC Asn	CTC Leu	TAC Tyr 375	AGT Ser	AGC Ser	CTG Leu	CCC Pro	CTG Leu 380	ACC Thr	AAG Lys	CGT Arg	GAG Glu	1152
GAG Glu 385	GTA Val	GAG Glu	AAA Lys	CTG Leu	CTC Leu 390	AAC Asn	GGG Gly	GAT Asp	ACC Thr	TGG Trp 395	CGA Arg	CAT His	CTG Leu	GCA Ala	GGC Gly 400	1200
GAG Glu	CTG Leu	GGT Gly	TAC Tyr	CAG Gln 405	CCT Pro	GAA Glu	CAT His	ATA Ile	GAC Asp 410	TCC Ser	TTT Phe	ACC Thr	CAC His	GAG Glu 415	GCC Ala	1248
TGC Cys	CCA Pro	GTG Val	CGA Arg 420	GCC Ala	CTG Leu	CTG Leu	GCC Ala	AGC Ser 425	TGG Trp	GGT Gly	GCC Ala	CAG Gln	GAC Asp 430	AGT Ser	GCA Ala	1296
ACG Thr	CTT Leu	GAT Asp 435	GCC Ala	CTT Leu	TTA Leu	GCC Ala	GCC Ala 440	CTG Leu	CGA Arg	CGC Arg	ATC Ile	CAG Gln 445	AGA Arg	GCT Ala	GAC Asp	1344
ATT Ile	GTG Val 450	GAG Glu	AGT Ser	CTA Leu	TGC Cys	AGC Ser 455	GAG Glu	TCC Ser	ACT Thr	GCC Ala	ACA Thr 460	TCC Ser	CCA Pro	GTG Val	TGA *	1392

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ACT Thr 465	CAC His	AGA Arg	CTG Leu	GGA Gly	GCC Ala 470	CCT Pro	GTC Val	CTG Leu	TCC Ser	CAC His 475	ATT Ile	CCG Pro	ACG Thr	ACT Thr	GAT Asp 480	1440
GTT Val	CTA Leu	GCC Ala	AGC Ser	CCC Pro 485	CAC His	AGA Arg	GCT Ala	GCC Ala	CCC Pro 490	TCT Ser	CCC Pro	TCG Ser	GGG Gly	ATG Met 495	GCC Ala	1488
CAA Gln	CGG Arg	TCA Ser	GAA Glu 500	CGG Arg	AGC Ser	ATC Ile	TCT Ser	GTG Val 505	CAG Gln	GGC Gly	CTC Leu	TGT Cys 510	GTT Val 510	CCC Pro	ACT Thr	1536
CCT Pro	GAC Asp	TCC Ser 515	GTT Val	GCT Ala	GCT Ala	CCC Pro	GAG Glu 520	GGG Gly	GCC Ala	CTT Leu	GCT Ala	TCT Ser 525	GAC Asp	CAC His	CCT Pro	1584
CTC Leu 530	CTC Leu	AGC Ser	AAG Lys	AGA Arg	GAG Glu 535	AGA Arg	GAG Glu	GAC Asp	CAC His	CCG Pro	AGC Ser 540	CTG Leu	ACT Thr	TGC Cys	TCC Ser	1632
ATT Ile 545	TCC Ser	ATC Ile	TCA Ser	GGC Gly	CTT Leu 550	TCC Ser	TTC Phe	CTT Leu	TCT Ser	ACA Thr 555	CAT His	TAG *	CTG Leu	TGT Cys	CAG Gln 560	1680
ATC Ile	TGG Trp	GGG Gly	TTT Phe	GAC Asp 565	ACT Thr	AGG Arg	AGA Arg	AGG Arg	GAG Glu 570	CGG Arg	GGG Gly	CAC His	CCC Pro	TAA *	GAC Asp 575	1728
TCA Ser	GGA Gly	GGT Gly	ACT Thr 580	GAA Glu	GAA Glu	CCA Pro	GAG Glu	CCA Pro 585	TGG Trp	ACT Thr	CCA Pro	CAC His	TGT Cys 590	GAA Glu	CCG Pro	1776
GAG Glu	AAC Asn 595	AAG Lys	GGG Gly	CGG Arg	GGC Gly	ATT Ile	GTG Val 600	GTA Val	GGC Gly	TAG *	ACC Thr	TTC Phe 605	CTT Leu	AGC Ser	CCC Pro	1824
TCC Ser 610	CTT Leu	CTC Leu	CCC Pro	TCT Ser	GGC Gly	CAA Gln 615	AGA Arg	AGA Arg	GGA Gly	TTA Leu	CGG Arg 620	ACC Thr	TAT Tyr	CTG Leu	AGC Ser	1872
TGA * 625	AAG Lys	CAG Gln	GTT Val	TGG Trp	AAC Asn 630	CCA Pro	GCC Ala	CAC His	ACT Thr	TCT Ser 635	CTC Leu	TCA Ser	CAC His	ACA Thr	GGA Gly 640	1920
TGG Trp	TAA *	AAC Asn	CCA Pro	GAG Glu 645	AAA Lys	GGC Gly	AGG Arg	GAC Asp	TGA * 650	CCT Pro	AGG Arg	CCA Pro	CCC Pro	AAC Asn 655	CAC His	1968
AGG Arg	AAG Lys	AAC Asn	AAA Lys 660	TGA *	AGG Arg	CTG Leu	ATA Ile	CAC His 665	TCC Ser	GTT Val	TCT Ser	GAA Glu 670	TGA *	GGG Gly	CGT Arg	2016
CAA Gln	GTG Val	TGC Cys 675	TTG Leu	TTG Leu	ACA Thr	GGG Gly	ATG Met 680	GCG Ala	TGA *	CTT Leu	TCA Ser	GGG Gly 685	AAA Lys	TAT Tyr	CTG Leu	2064
GAA Glu 690	GCC Ala	ATG Met	TCT Ser	GCC Ala	CCG Pro	CCC Pro 695	TCA Ser	ACC Thr	ACT Thr	TCC Ser	AGG Arg 700	CCC Pro	CTA Leu	CCC Pro	AAC Asn	2112
CCT Pro 705	TGT Cys	GCA Ala	GAT Asp	GAA Glu	CTG Leu 710	TTT Phe	GTT Val	CAA Gln	GGG Gly	CTG Leu 715	GTC Val	CAT His	TGG Trp	TCT Ser	ATT Ile 720	2160
CTG Leu	ATG Met	GAG Glu	TCA Ser	AGC Ser 725	TAA *	GGG Gly	CTC Leu	AGG Arg	CTT Leu 730	ATC Ile	CAT His	AAG Lys	GCA Ala	TTT Phe 735	GTG Val	2208

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GAG Glu	AGA Arg	TGA *	ATC Ile 740	TGT Cys	TAG *	TGC Cys	GCT Ala	CAT His 745	TCT Ser	TGG Trp	CAT His	AAG Lys	CCT Pro 750	GAA Glu	GCC Ala	2256
AAC Asn	ACG Thr	GCC Ala 755	CTT Leu	AAT Asn	GTC Val	AGC Ser	CCT Pro 760	CGG Arg	GGT Gly	CAG Gln	GAA Glu	CCA Pro 765	AGG Arg	ACT Thr	CCC Pro	2304
ACC Thr 770	CCA Pro	CAA Gln	TCC Ser	AAC Asn	ACT Thr	ATA Ile 775	CTA Leu	CAT His	TAC Tyr	ACA Thr	CAC His 780	ACA Thr	CAC His	ACA Thr	CAC His	2352
ACA Thr 785	CAC His	ACA Thr	CAC His	ACA Thr	CAC His 790	ACA Thr	CAC His	ACA Thr	GAT Asp	ATC Ile 795	TTG Leu	CTT Leu	TTC Phe	TCC Ser	CCA Pro 800	2400
TGG Trp	CTC Leu	TTT Phe	TGG Trp	GGC Gly 805	TGA *	GAC Asp	TAG *	ATC Ile 810	CTG Leu	CTG Leu	GGA Gly	GTC Val	ACT Thr	GCC Ala 815	AGT Ser	2448
GAG Glu	AGA Arg	TCC Ser	GGA Gly 820	GGG Gly	GAC Asp	AGA Arg	GCT Ala	GAG Glu 825	CTT Leu	CAT His	GGG Gly	GCT Ala	GTC Val 830	TTC Phe	CTC Leu	2496
GCC Ala	CCC Pro	GGG Gly 835	TCT Ser	GGC Gly	AGG Arg	CCA Pro	AGA Arg 840	ATG Met	ACT Thr	GCA Ala	TCT Ser	GAG Glu 845	CTG Leu	GTG Val	TCT Ser	2544
GTC Val 850	TTC Phe	CAA Gln	TGG Trp	CCT Pro	GTG Val	CGT Arg 855	GGA Gly	GGA Gly	AAT Asn	GCT Ala	CCC Pro 860	ACT Thr	CCT Pro	CCC Pro	CTT Leu	2592
CTT Leu 865	GAA Glu	GCT Ala	GCC Ala	CCC Pro	AGA Arg 870	AGA Arg	CTA Leu	CAG Gln	TGC Cys	AAA Lys 875	AGA Arg	GCA Ala	GAC Asp	TGG Trp	TGT Cys 880	2640
GAG Glu	AAC Asn	ACA Thr	AGA Arg	AAA Lys 885	AGC Ser	AGA Arg	TGC Cys	TGG Trp	CCC Pro 890	TGC Cys	AGT Ser	CTG Leu	TGG Trp	CAG Gln 895	CTT Leu	2688
TCT Ser	CCT Pro	CAG Gln 900	CTT Leu	CAA Gln	GGC Gly	CCC Pro	TGC Cys	AAA Lys 905	GGA Gly	CGG Arg	ATT Ile	TCC Ser	TGA *	GCA Ala 910	CGG Arg	2736
CCA Pro	GGA Gly	AGG Arg 915	GGC Gly	AAG Lys	AGG Arg	GTT Val	CGG Arg 920	TTC Phe	AGT Ser	GGC Gly	GCT Ala	TTC Phe 925	TCC Ser	CGG Arg	CTC Leu	2784
CTT Leu 930	GGC Gly	CTG Leu	TTC Phe	TGT Cys	TTT Phe	GCT Ala 935	TGC Cys	TGT Cys	TGG Trp	AAT Asn	GAG Glu 940	TGG Trp	GCA Ala	CCC Pro	CCT Pro	2832
CTA Leu 945	TTT Phe	AGC Ser	ATG Met	AAG Lys	GAG Glu 950	CCC Pro	CAG Gln	GCA Ala	GGG Gly	TAT Tyr 955	GCA Ala	CAG Gln	ACT Thr	GAC Asp	CAC His 960	2880
CAT His	CCC Pro	TCC Ser	CCA Pro	CCC Pro 965	AGG Arg	GTC Val	CAC His	CCA Pro	ACC Thr 970	CGG Arg	TGA *	AGA Arg	GAC Asp	CAG Gln 975	GAG Glu	2928
CAT His	TGT Cys	ACG Thr	CAT His 980	ACG Thr	CGG Arg	GTG Val	GTA Val	TTT Phe 985	TTA Leu	TGG Trp	ACC Thr	CCA Pro	ATC Ile 990	TGC Cys	AAT Asn	2976
TCC Ser	CAG Gln	ACA Thr 995	CCT Pro	GGG Gly	AAG Lys	TGG Trp	GAC Asp 1000	ATT Ile	CTT Leu	TGT Cys	GTA Val	TTT Phe 1005	ATT Ile	TTC Phe	CTC Leu	3024

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CCC	AGG	AGC	TGG	GGA	GTG	GTG	GGG	GGC	TGC	AGG	TAC	GGT	TTA	GCA	TGT	3072
Pro	Arg	Ser	Trp	Gly	Val	Val	Gly	Gly	Cys	Arg	Tyr	Gly	Leu	Ala	Cys	
	1010						1015				1020					
GTT	TGG	TTC	TGG	GGG	TCT	CTC	CAG	CCT	TGT	TTT	GGG	CCA	AGT	TGG	AAC	3120
Val	Trp	Phe	Trp	Gly	Ser	Leu	Gln	Pro	Cys	Phe	Gly	Pro	Ser	Trp	Asn	
1025					1030				1035						1040	
CTC	TGG	CCC	TCC	AGC	TGG	TGA	CTA	TGA	ACT	CCA	GAC	CCC	TTC	GTG	CTC	3168
Leu	Trp	Pro	Ser	Ser	Trp	*	Leu	*	Thr	Pro	Asp	Pro	Phe	Val	Leu	
				1045					1050					1055		
CCC	GAC	GCC	TTC	CCC	TTG	CAT	CCT	GTG	TAA	CCA	TTT	CGT	TGG	GCC	CTC	3216
Pro	Asp	Ala	Phe	Pro	Leu	His	Pro	Val	*	Pro	Phe	Arg	Trp	Ala	Leu	
			1060					1065					1070			
CCA	AAA	CCT	ACA	CAT	AAA	ACA	TAC	AGG	AGG	ACC	ATT	AAA	TTG	GC		3260
Pro	Lys	Pro	Thr	His	Lys	Thr	Tyr	Arg	Arg	Thr	Ile	Lys	Leu			
	1075						1080					1085				

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(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1086 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Thr 1	Ala	Pro	Ala	Gly 5	Ser	Arg	Arg	Trp	Ser 10	Ala	Ser	Gln	Phe	Ser 15	Ser
Ala	Gln	His	His 20	Arg	Ser	Ala	Glu	Arg 25	Thr	Glu	Leu	Glu	Ala 30	Glu	Arg
*	Arg	Arg 35	Arg	Arg	Ala	Met	Arg 40	Arg	Ala	Gly	Ala	Ala 45	Cys	Ser	Ala
Met	Asp 50	Arg	Leu	Arg	Leu	Leu 55	Leu	Leu	Leu	Ile	Leu 60	Gly	Val	Ser	Ser
Gly 65	Gly	Ala	Lys	Glu	Thr 70	Cys	Ser	Thr	Gly	Leu 75	Tyr	Thr	His	Ser	Gly 80
Glu	Cys	Cys	Lys	Ala 85	Cys	Asn	Leu	Gly	Glu 90	Gly	Val	Ala	Gln	Pro 95	Cys
Gly	Ala	Asn	Gln 100	Thr	Val	Cys	Glu	Pro 105	Cys	Leu	Asp	Asn	Val 110	Thr	Phe
Ser	Asp	Val 115	Val	Ser	Ala	Thr	Glu 120	Pro	Cys	Lys	Pro	Cys 125	Thr	Glu	Cys
Leu	Gly 130	Leu	Gln	Ser	Met	Ser 135	Ala	Pro	Cys	Val	Glu 140	Ala	Asp	Asp	Ala
Val 145	Cys	Arg	Cys	Ala	Tyr 150	Gly	Tyr	Tyr	Gln	Asp 155	Glu	Glu	Thr	Gly	His 160
Cys	Glu	Ala	Cys	Ser 165	Val	Cys	Glu	Val	Gly 170	Ser	Gly	Leu	Val	Phe 175	Ser
Cys	Gln	Asp	Lys 180	Gln	Asn	Thr	Val	Cys 185	Glu	Glu	Cys	Pro	Glu 190	Gly	Thr
Tyr	Ser	Asp 195	Glu	Ala	Asn	His	Val 200	Asp	Pro	Cys	Leu	Pro 205	Cys	Thr	Val
Cys	Glu 210	Asp	Thr	Glu	Arg	Gln 215	Leu	Arg	Glu	Cys	Thr 220	Pro	Trp	Ala	Asp
Ala 225	Glu	Cys	Glu	Glu	Ile 230	Pro	Gly	Arg	Trp	Ile 235	Pro	Arg	Ser	Thr	Pro 240
Pro	Glu	Gly	Ser	Asp 245	Ser	Thr	Ala	Pro	Ser 250	Thr	Gln	Glu	Pro	Glu 255	Val
Pro	Pro	Glu	Gln 260	Asp	Leu	Val	Pro	Ser 265	Thr	Val	Ala	Asp	Met 270	Val	Thr
Thr	Val	Met 275	Gly	Ser	Ser	Gln	Pro 280	Val	Val	Thr	Arg	Gly 285	Thr	Thr	Asp
Asn	Leu 290	Ile	Pro	Val	Tyr	Cys 295	Ser	Ile	Leu	Ala	Ala 300	Val	Val	Val	Gly

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Leu Val Ala Tyr Ile Ala Phe Lys Arg Trp Asn Ser Cys Lys Gln Asn
 305 310 315 320
 Lys Gln Gly Ala Asn Ser Arg Pro Val Asn Gln Thr Pro Pro Pro Glu
 325 330 335
 Gly Glu Lys Leu His Ser Asp Ser Gly Ile Ser Val Asp Ser Gln Ser
 340 345 350
 Leu His Asp Gln Gln Thr His Thr Gln Thr Ala Ser Gly Gln Ala Leu
 355 360 365
 Lys Gly Asp Gly Asn Leu Tyr Ser Ser Leu Pro Leu Thr Lys Arg Glu
 370 375 380
 Glu Val Glu Lys Leu Leu Asn Gly Asp Thr Trp Arg His Leu Ala Gly
 385 390 395 400
 Glu Leu Gly Tyr Gln Pro Glu His Ile Asp Ser Phe Thr His Glu Ala
 405 410 415
 Cys Pro Val Arg Ala Leu Leu Ala Ser Trp Gly Ala Gln Asp Ser Ala
 420 425 430
 Thr Leu Asp Ala Leu Leu Ala Ala Leu Arg Arg Ile Gln Arg Ala Asp
 435 440 445
 Ile Val Glu Ser Leu Cys Ser Glu Ser Thr Ala Thr Ser Pro Val *
 450 455 460
 Thr His Arg Leu Gly Ala Pro Val Leu Ser His Ile Pro Thr Thr Asp
 465 470 475 480
 Val Leu Ala Ser Pro His Arg Ala Ala Pro Ser Pro Ser Gly Met Ala
 485 490 495
 Gln Arg Ser Glu Arg Ser Ile Ser Val Gln Gly Leu Cys Val Pro Thr
 500 505 510
 Pro Asp Ser Val Ala Ala Pro Glu Gly Ala Leu Ala Ser Asp His Pro
 515 520 525
 Leu Leu Ser Lys Arg Glu Arg Glu Asp His Pro Ser Leu Thr Cys Ser
 530 535 540
 Ile Ser Ile Ser Gly Leu Ser Phe Leu Ser Thr His * Leu Cys Gln
 545 550 555 560
 Ile Trp Gly Phe Asp Thr Arg Arg Arg Glu Arg Gly His Pro * Asp
 565 570 575
 Ser Gly Gly Thr Glu Glu Pro Glu Pro Trp Thr Pro His Cys Glu Pro
 580 585 590
 Glu Asn Lys Gly Arg Gly Ile Val Val Gly * Thr Phe Leu Ser Pro
 595 600 605
 Ser Leu Leu Pro Ser Gly Gln Arg Arg Gly Leu Arg Thr Tyr Leu Ser
 610 615 620
 * Lys Gln Val Trp Asn Pro Ala His Thr Ser Leu Ser His Thr Gly
 625 630 635 640
 Trp * Asn Pro Glu Lys Gly Arg Asp * Pro Arg Pro Pro Asn His
 645 650 655

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Arg Lys Asn Lys * Arg Leu Ile His Ser Val Ser Glu * Gly Arg
 660 665 670
 Gln Val Cys Leu Leu Thr Gly Met Ala * Leu Ser Gly Lys Tyr Leu
 675 680 685
 Glu Ala Met Ser Ala Pro Pro Ser Thr Thr Ser Arg Pro Leu Pro Asn
 690 695 700
 Pro Cys Ala Asp Glu Leu Phe Val Gln Gly Leu Val His Trp Ser Ile
 705 710 715 720
 Leu Met Glu Ser Ser * Gly Leu Arg Leu Ile His Lys Ala Phe Val
 725 730 735
 Glu Arg * Ile Cys * Cys Ala His Ser Trp His Lys Pro Glu Ala
 740 745 750
 Asn Thr Ala Leu Asn Val Ser Pro Arg Gly Gln Glu Pro Arg Thr Pro
 755 760 765
 Thr Pro Gln Ser Asn Thr Ile Leu His Tyr Thr His Thr His Thr His
 770 775 780
 Thr His Thr His Thr His Thr His Thr Asp Ile Leu Leu Phe Ser Pro
 785 790 795 800
 Trp Leu Phe Trp Gly * Asp * Ile Leu Leu Gly Val Thr Ala Ser
 805 810 815
 Glu Arg Ser Gly Gly Asp Arg Ala Glu Leu His Gly Ala Val Phe Leu
 820 825 830
 Ala Pro Gly Ser Gly Arg Pro Arg Met Thr Ala Ser Glu Leu Val Ser
 835 840 845
 Val Phe Gln Trp Pro Val Arg Gly Gly Asn Ala Pro Thr Pro Pro Leu
 850 855 860
 Leu Glu Ala Ala Pro Arg Arg Leu Gln Cys Lys Arg Ala Asp Trp Cys
 865 870 875 880
 Glu Asn Thr Arg Lys Ser Arg Cys Trp Pro Cys Ser Leu Trp Gln Leu
 885 890 895
 Ser Pro Gln Leu Gln Gly Pro Cys Lys Gly Arg Ile Ser * Ala Arg
 900 905 910
 Pro Gly Arg Gly Lys Arg Val Arg Phe Ser Gly Ala Phe Ser Arg Leu
 915 920 925
 Leu Gly Leu Phe Cys Phe Ala Cys Cys Trp Asn Glu Trp Ala Pro Pro
 930 935 940
 Leu Phe Ser Met Lys Glu Pro Gln Ala Gly Tyr Ala Gln Thr Asp His
 945 950 955 960
 His Pro Ser Pro Pro Arg Val His Pro Thr Arg * Arg Asp Gln Glu
 965 970 975
 His Cys Thr His Thr Arg Val Val Phe Leu Trp Thr Pro Ile Cys Asn
 980 985 990
 Ser Gln Thr Pro Gly Lys Trp Asp Ile Leu Cys Val Phe Ile Phe Leu
 995 1000 1005

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Pro Arg Ser Trp Gly Val Val Gly Gly Cys Arg Tyr Gly Leu Ala Cys
1010                               1015                1020

Val Trp Phe Trp Gly Ser Leu Gln Pro Cys Phe Gly Pro Ser Trp Asn
1025                               1030                1035                1040

Leu Trp Pro Ser Ser Trp * Leu * Thr Pro Asp Pro Phe Val Leu
                               1045                1050                1055

Pro Asp Ala Phe Pro Leu His Pro Val * Pro Phe Arg Trp Ala Leu
                               1060                1065                1070

Pro Lys Pro Thr His Lys Thr Tyr Arg Arg Thr Ile Lys Leu
1075                               1080                1085

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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 867 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..867

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ACA GCT CCG GCG GGC AGC AGG CGC TGG AGC GCA TCG CAG TTC AGC TCA	48
Thr Ala Pro Ala Gly Ser Arg Arg Trp Ser Ala Ser Gln Phe Ser	
1 5 10 15	
GCG CAG CAC CAT CGG TCT GCG GAG CGG ACT GAG CTA GAA GCG GAG CGC	96
Ala Gln His His Arg Ser Ala Glu Arg Thr Glu Leu Glu Ala Glu Arg	
20 25 30	
TGA CGC CGG AGG CGT GCA ATG AGG AGG GCA GGT GCT GCC TGC AGC GCC	144
* Arg Arg Arg Arg Ala Met Arg Arg Ala Gly Ala Ala Cys Ser Ala	
35 40 45	
ATG GAC CGG CTG CGC CTG CTG CTG CTG CTG ATT CTA GGG GTG TCC TCT	192
Met Asp Arg Leu Arg Leu Leu Leu Leu Ile Leu Gly Val Ser Ser	
50 55 60	
GGA GGT GCC AAG GAG ACA TGT TCC ACA GGC CTG TAC ACC CAC AGC GGA	240
Gly Gly Ala Lys Glu Thr Cys Ser Thr Gly Leu Tyr Thr His Ser Gly	
65 70 75 80	
GAG TGC TGC AAA GCC TGC AAC TTG GGC GAA GGC GTG GCC CAG CCC TGC	288
Glu Cys Cys Lys Ala Cys Asn Leu Gly Glu Gly Val Ala Gln Pro Cys	
85 90 95	
GGA GCC AAC CAG ACC GTG TGT GAA CCC TGC CTG GAC AAT GTT ACA TTC	336
Gly Ala Asn Gln Thr Val Cys Glu Pro Cys Leu Asp Asn Val Thr Phe	
100 105 110	
TCC GAT GTG GTG AGC GCC ACT GAG CCG TGC AAG CCG TGC ACC GAG TGC	384
Ser Asp Val Val Ser Ala Thr Glu Pro Cys Lys Pro Cys Thr Glu Cys	
115 120 125	

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CTG	GGC	CTG	CAG	AGC	ATG	TCC	GCT	CCC	TGT	GTG	GAG	GCA	GAC	GAT	GCA	432
Leu	Gly	Leu	Gln	Ser	Met	Ser	Ala	Pro	Cys	Val	Glu	Ala	Asp	Asp	Ala	
	130					135					140					
GTG	TGC	AGA	TGT	GCC	TAT	GGC	TAC	TAC	CAG	GAC	GAG	GAG	ACT	GGC	CAC	480
Val	Cys	Arg	Cys	Ala	Tyr	Gly	Tyr	Tyr	Gln	Asp	Glu	Glu	Thr	Gly	His	
	145				150					155					160	
TGT	GAG	GCT	TGC	AGC	GTG	TGC	GAG	GTG	GGC	TCG	GGA	CTC	GTG	TTC	TCC	528
Cys	Glu	Ala	Cys	Ser	Val	Cys	Glu	Val	Gly	Ser	Gly	Leu	Val	Phe	Ser	
				165					170					175		
TGC	CAG	GAC	AAA	CAG	AAC	ACA	GTG	TGT	GAA	GAG	TGC	CCA	GAG	GGC	ACA	576
Cys	Gln	Asp	Lys	Gln	Asn	Thr	Val	Cys	Glu	Glu	Cys	Pro	Glu	Gly	Thr	
			180					185					190			
TAC	TCA	GAC	GAA	GCC	AAC	CAC	GTG	GAC	CCG	TGC	CTA	CCC	TGC	ACG	GTG	624
Tyr	Ser	Asp	Glu	Ala	Asn	His	Val	Asp	Pro	Cys	Leu	Pro	Cys	Thr	Val	
		195					200					205				
TGC	GAG	GAC	ACT	GAG	CGC	CAG	TTA	CGC	GAG	TGC	ACG	CCC	TGG	GCT	GAT	672
Cys	Glu	Asp	Thr	Glu	Arg	Gln	Leu	Arg	Glu	Cys	Thr	Pro	Trp	Ala	Asp	
	210					215					220					
GCT	GAA	TGC	GAA	GAG	ATC	CCT	GGT	CGA	TGG	ATC	CCA	AGG	TCT	ACG	CCC	720
Ala	Glu	Cys	Glu	Glu	Ile	Pro	Gly	Arg	Trp	Ile	Pro	Arg	Ser	Thr	Pro	
	225				230					235					240	
CCG	GAG	GGC	TCC	GAC	AGC	ACA	GCG	CCC	AGC	ACC	CAG	GAG	CCT	GAG	GTT	768
Pro	Glu	Gly	Ser	Asp	Ser	Thr	Ala	Pro	Ser	Thr	Gln	Glu	Pro	Glu	Val	
				245					250					255		
CCT	CCA	GAG	CAA	GAC	CTT	GTA	CCC	AGT	ACA	GTG	GCG	GAT	ATG	GTG	ACC	816
Pro	Pro	Glu	Gln	Asp	Leu	Val	Pro	Ser	Thr	Val	Ala	Asp	Met	Val	Thr	
			260				265						270			
ACT	GTG	ATG	GGC	AGC	TCC	CAG	CCT	GTA	GTG	ACC	CGC	GGC	ACC	ACC	GAC	864
Thr	Val	Met	Gly	Ser	Ser	Gln	Pro	Val	Val	Thr	Arg	Gly	Thr	Thr	Asp	
		275					280					285				
AAC																867
Asn																

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 289 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Thr Ala Pro Ala Gly Ser Arg Arg Trp Ser Ala Ser Gln Phe Ser Ser
 1 5 10 15

Ala Gln His His Arg Ser Ala Glu Arg Thr Glu Leu Glu Ala Glu Arg
 20 25 30

* Arg Arg Arg Arg Ala Met Arg Arg Ala Gly Ala Ala Cys Ser Ala
 35 40 45

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Met	Asp	Arg	Leu	Arg	Leu	Leu	Leu	Leu	Leu	Ile	Leu	Gly	Val	Ser	Ser
	50					55					60				
Gly	Gly	Ala	Lys	Glu	Thr	Cys	Ser	Thr	Gly	Leu	Tyr	Thr	His	Ser	Gly
65					70					75					80
Glu	Cys	Cys	Lys	Ala	Cys	Asn	Leu	Gly	Glu	Gly	Val	Ala	Gln	Pro	Cys
			85						90					95	
Gly	Ala	Asn	Gln	Thr	Val	Cys	Glu	Pro	Cys	Leu	Asp	Asn	Val	Thr	Phe
			100					105					110		
Ser	Asp	Val	Val	Ser	Ala	Thr	Glu	Pro	Cys	Lys	Pro	Cys	Thr	Glu	Cys
		115					120					125			
Leu	Gly	Leu	Gln	Ser	Met	Ser	Ala	Pro	Cys	Val	Glu	Ala	Asp	Asp	Ala
130						135					140				
Val	Cys	Arg	Cys	Ala	Tyr	Gly	Tyr	Tyr	Gln	Asp	Glu	Glu	Thr	Gly	His
145					150					155					160
Cys	Glu	Ala	Cys	Ser	Val	Cys	Glu	Val	Gly	Ser	Gly	Leu	Val	Phe	Ser
				165					170					175	
Cys	Gln	Asp	Lys	Gln	Asn	Thr	Val	Cys	Glu	Glu	Cys	Pro	Glu	Gly	Thr
			180					185					190		
Tyr	Ser	Asp	Glu	Ala	Asn	His	Val	Asp	Pro	Cys	Leu	Pro	Cys	Thr	Val
		195					200					205			
Cys	Glu	Asp	Thr	Glu	Arg	Gln	Leu	Arg	Glu	Cys	Thr	Pro	Trp	Ala	Asp
	210					215					220				
Ala	Glu	Cys	Glu	Glu	Ile	Pro	Gly	Arg	Trp	Ile	Pro	Arg	Ser	Thr	Pro
225					230					235					240
Pro	Glu	Gly	Ser	Asp	Ser	Thr	Ala	Pro	Ser	Thr	Gln	Glu	Pro	Glu	Val
				245					250					255	
Pro	Pro	Glu	Gln	Asp	Leu	Val	Pro	Ser	Thr	Val	Ala	Asp	Met	Val	Thr
			260					265					270		
Thr	Val	Met	Gly	Ser	Ser	Gln	Pro	Val	Val	Thr	Arg	Gly	Thr	Thr	Asp
		275					280					285			

Asn

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(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 66 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..66

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTC	ATT	CCT	GTC	TAT	TGC	TCC	ATC	TTG	GCT	GCT	GTG	GTC	GTG	GGC	CTT	48
Leu	Ile	Pro	Val	Tyr	Cys	Ser	Ile	Leu	Ala	Ala	Val	Val	Val	Gly	Leu	
1				5				10						15		
GTG	GCC	TAT	ATT	GCT	TTC											66
Val	Ala	Tyr	Ile	Ala	Phe											
				20												

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Leu	Ile	Pro	Val	Tyr	Cys	Ser	Ile	Leu	Ala	Ala	Val	Val	Val	Gly	Leu
1				5				10						15	
Val	Ala	Tyr	Ile	Ala	Phe										
				20											

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 105 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..105

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAG	AGG	TGG	AAC	AGC	TGC	AAA	CAA	AAT	AAA	CAA	GGC	GCC	AAC	AGC	CGC	48
Lys	Arg	Trp	Asn	Ser	Cys	Lys	Gln	Asn	Lys	Gln	Gly	Ala	Asn	Ser	Arg	
1				5				10						15		

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CCC GTG AAC CAG ACG CCC CCA CCG GAG GGA GAG AAA CTG CAC AGC GAC 96
Pro Val Asn Gln Thr Pro Pro Pro Glu Gly Glu Lys Leu His Ser Asp
20 25 30

AGT GGC ATC 105
Ser Gly Ile
35

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Lys Arg Trp Asn Ser Cys Lys Gln Asn Lys Gln Gly Ala Asn Ser Arg
1 5 10 15

Pro Val Asn Gln Thr Pro Pro Pro Glu Gly Glu Lys Leu His Ser Asp
20 25 30

Ser Gly Ile
35

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2222 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..2222

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TCT	GTG	GAC	AGC	CAG	AGC	CTG	CAC	GAC	CAG	CAG	ACC	CAT	ACG	CAG	ACT	48
Ser	Val	Asp	Ser	Gln	Ser	Leu	His	Asp	Gln	Gln	Thr	His	Thr	Gln	Thr	
1				5					10					15		

GCC TCA GGC CAG GCC CTC AAG GGT GAT GGC AAC CTC TAC AGT AGC CTG 96
Ala Ser Gly Gln Ala Leu Lys Gly Asp Gly Asn Leu Tyr Ser Ser Leu
20 25 30

CCC CTG ACC AAG CGT GAG GAG GTA GAG AAA CTG CTC AAC GGG GAT ACC 144
Pro Leu Thr Lys Arg Glu Glu Val Glu Lys Leu Leu Asn Gly Asp Thr
35 40 45

TGG CGA CAT CTG GCA GGC GAG CTG GGT TAC CAG CCT GAA CAT ATA GAC 192
Trp Arg His Leu Ala Gly Glu Leu Gly Tyr Gln Pro Glu His Ile Asp
50 55 60



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TCC Ser 65	TTT Phe	ACC Thr	CAC His	GAG Glu	GCC Ala 70	TGC Cys	CCA Pro	GTG Val	CGA Arg	GCC Ala 75	CTG Leu	CTG Leu	GCC Ala	AGC Ser	TGG Trp 80	240
GGT Gly	GCC Ala	CAG Gln	GAC Asp	AGT Ser 85	GCA Ala	ACG Thr	CTT Leu	GAT Asp	GCC Ala 90	CTT Leu	TTA Leu	GCC Ala	GCC Ala	CTG Leu 95	CGA Arg	288
CGC Arg	ATC Ile	CAG Gln	AGA Arg 100	GCT Ala	GAC Asp	ATT Ile	GTG Val	GAG Glu 105	AGT Ser	CTA Leu	TGC Cys	AGC Ser	GAG Glu 110	TCC Ser	ACT Thr	336
GCC Ala	ACA Thr	TCC Ser 115	CCA Pro	GTG Val	TGA *	ACT Thr 120	CAC His	AGA Arg	CTG Leu	GGA Gly	GCC Ala	CCT Pro 125	GTC Val	CTG Leu	TCC Ser	384
CAC His 130	ATT Ile	CCG Pro	ACG Thr	ACT Thr	GAT Asp	GTT Val 135	CTA Leu	GCC Ala	AGC Ser	CCC Pro	CAC His 140	AGA Arg	GCT Ala	GCC Ala	CCC Pro	432
TCT Ser 145	CCC Pro	TCG Ser	GGG Gly	ATG Met	GCC Ala 150	CAA Gln	CGG Arg	TCA Ser	GAA Glu	CGG Arg 155	AGC Ser	ATC Ile	TCT Ser	GTG Val	CAG Gln 160	480
GGC Gly	CTC Leu	TGT Cys	GTT Val	CCC Pro 165	ACT Thr	CCT Pro	GAC Asp	TCC Ser	GTT Val 170	GCT Ala	GCT Ala	CCC Pro	GAG Glu	GGG Gly 175	GCC Ala	528
CTT Leu	GCT Ala	TCT Ser	GAC Asp 180	CAC His	CCT Pro	CTC Leu	CTC Leu	AGC Ser 185	AAG Lys	AGA Arg	GAG Glu	AGA Arg	GAG Glu 190	GAC Asp	CAC His	576
CCG Pro	AGC Ser	CTG Leu 195	ACT Thr	TGC Cys	TCC Ser	ATT Ile	TCC Ser 200	ATC Ile	TCA Ser	GGC Gly	CTT Leu	TCC Ser 205	TTC Phe	CTT Leu	TCT Ser	624
ACA Thr 210	CAT His	TAG *	CTG Leu	TGT Cys	CAG Gln	ATC Ile 215	TGG Trp	GGG Gly	TTT Phe	GAC Asp	ACT Thr 220	AGG Arg	AGA Arg	AGG Arg	GAG Glu	672
CGG Arg 225	GGG Gly	CAC His	CCC Pro	TAA *	GAC Asp 230	TCA Ser	GGA Gly	GGT Gly	ACT Thr	GAA Glu 235	GAA Glu	CCA Pro	GAG Glu	CCA Pro	TGG Trp 240	720
ACT Thr	CCA Pro	CAC His	TGT Cys	GAA Glu 245	CCG Pro	GAG Glu	AAC Asn	AAG Lys	GGG Gly 250	CGG Arg	GGC Gly	ATT Ile	GTG Val	GTA Val 255	GGC Gly	768
TAG *	ACC Thr	TTC Phe	CTT Leu 260	AGC Ser	CCC Pro	TCC Ser	CTT Leu 265	CTC Leu	CCC Pro	TCT Ser	GGC Gly	CAA Gln	AGA Arg 270	AGA Arg	GGA Gly	816
TTA Leu	CGG Arg	ACC Thr 275	TAT Tyr	CTG Leu	AGC Ser	TGA *	AAG Lys 280	CAG Gln	GTT Val	TGG Trp	AAC Asn	CCA Pro 285	GCC Ala	CAC His	ACT Thr	864
TCT Ser 290	CTC Leu	TCA Ser	CAC His	ACA Thr	GGA Gly	TGG Trp 295	TAA *	AAC Asn	CCA Pro	GAG Glu	AAA Lys 300	GGC Gly	AGG Arg	GAC Asp	TGA *	912
CCT Pro 305	AGG Arg	CCA Pro	CCC Pro	AAC Asn	CAC His 310	AGG Arg	AAG Lys	AAC Asn	AAA Lys	TGA *	AGG Arg 315	CTG Leu	ATA Ile	CAC His	TCC Ser 320	960
GTT Val	TCT Ser	GAA Glu	TGA *	GGG Gly 325	CGT Arg	CAA Gln	GTG Val	TGC Cys	TTG Leu 330	TTG Leu	ACA Thr	GGG Gly	ATG Met	GCG Ala 335	TGA *	1008

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CTT	TCA	GGG	AAA	TAT	CTG	GAA	GCC	ATG	TCT	GCC	CCG	CCC	TCA	ACC	ACT	1056
Leu	Ser	Gly	Lys	Tyr	Leu	Glu	Ala	Met	Ser	Ala	Pro	Pro	Ser	Thr	Thr	
			340					345					350			
TCC	AGG	CCC	CTA	CCC	AAC	CCT	TGT	GCA	GAT	GAA	CTG	TTT	GTT	CAA	GGG	1104
Ser	Arg	Pro	Leu	Pro	Asn	Pro	Cys	Ala	Asp	Glu	Leu	Phe	Val	Gln	Gly	
		355					360					365				
CTG	GTC	CAT	TGG	TCT	ATT	CTG	ATG	GAG	TCA	AGC	TAA	GGG	CTC	AGG	CTT	1152
Leu	Val	His	Trp	Ser	Ile	Leu	Met	Glu	Ser	Ser	*	Gly	Leu	Arg	Leu	
	370					375					380					
ATC	CAT	AAG	GCA	TTT	GTG	GAG	AGA	TGA	ATC	TGT	TAG	TGC	GCT	CAT	TCT	1200
Ile	His	Lys	Ala	Phe	Val	Glu	Arg	*	Ile	Cys	*	Cys	Ala	His	Ser	
385					390					395					400	
TGG	CAT	AAG	CCT	GAA	GCC	AAC	ACG	GCC	CTT	AAT	GTC	AGC	CCT	CGG	GGT	1248
Trp	His	Lys	Pro	Glu	Ala	Asn	Thr	Ala	Leu	Asn	Val	Ser	Pro	Arg	Gly	
				405				410						415		
CAG	GAA	CCA	AGG	ACT	CCC	ACC	CCA	CAA	TCC	AAC	ACT	ATA	CTA	CAT	TAC	1296
Gln	Glu	Pro	Arg	Thr	Pro	Thr	Pro	Gln	Ser	Asn	Thr	Ile	Leu	His	Tyr	
			420					425					430			
ACA	CAC	ACA	CAC	ACA	CAC	ACA	CAC	ACA	CAC	ACA	CAC	ACA	CAC	ACA	GAT	1344
Thr	His	Thr	His	Thr	His	Thr	His	Thr	His	Thr	His	Thr	His	Thr	Asp	
		435					440					445				
ATC	TTG	CTT	TTC	TCC	CCA	TGG	CTC	TTT	TGG	GGC	TGA	GAC	TAG	ATC	CTG	1392
Ile	Leu	Leu	Phe	Ser	Pro	Trp	Leu	Phe	Trp	Gly	*	Asp	*	Ile	Leu	
	450					455					460					
CTG	GGA	GTC	ACT	GCC	AGT	GAG	AGA	TCC	GGA	GGG	GAC	AGA	GCT	GAG	CTT	1440
Leu	Gly	Val	Thr	Ala	Ser	Glu	Arg	Ser	Gly	Gly	Asp	Arg	Ala	Glu	Leu	
465				470						475					480	
CAT	GGG	GCT	GTC	TTC	CTC	GCC	CCC	GGG	TCT	GGC	AGG	CCA	AGA	ATG	ACT	1488
His	Gly	Ala	Val	Phe	Leu	Ala	Pro	Gly	Ser	Gly	Arg	Pro	Arg	Met	Thr	
				485				490						495		
GCA	TCT	GAG	CTG	GTG	TCT	GTC	TTC	CAA	TGG	CCT	GTG	CGT	GGA	GGA	AAT	1536
Ala	Ser	Glu	Leu	Val	Ser	Val	Phe	Gln	Trp	Pro	Val	Arg	Gly	Gly	Asn	
			500					505					510			
GCT	CCC	ACT	CCT	CCC	CTT	CTT	GAA	GCT	GCC	CCC	AGA	AGA	CTA	CAG	TGC	1584
Ala	Pro	Thr	Pro	Pro	Leu	Leu	Glu	Ala	Ala	Pro	Arg	Arg	Leu	Gln	Cys	
		515					520					525				
AAA	AGA	GCA	GAC	TGG	TGT	GAG	AAC	ACA	AGA	AAA	AGC	AGA	TGC	TGG	CCC	1632
Lys	Arg	Ala	Asp	Trp	Cys	Glu	Asn	Thr	Arg	Lys	Ser	Arg	Cys	Trp	Pro	
	530					535					540					
TGC	AGT	CTG	TGG	CAG	CTT	TCT	CCT	CAG	CTT	CAA	GGC	CCC	TGC	AAA	GGA	1680
Cys	Ser	Leu	Trp	Gln	Leu	Ser	Pro	Gln	Leu	Gln	Gly	Pro	Cys	Lys	Gly	
545				550						555					560	
CGG	ATT	TCC	TGA	GCA	CGG	CCA	GGA	AGG	GGC	AAG	AGG	GTT	CGG	TTC	AGT	1728
Arg	Ile	Ser	*	Ala	Arg	Pro	Gly	Arg	Gly	Lys	Arg	Val	Arg	Phe	Ser	
				565				570						575		
GGC	GCT	TTC	TCC	CGG	CTC	CTT	GGC	CTG	TTC	TGT	TTT	GCT	TGC	TGT	TGG	1776
Gly	Ala	Phe	Ser	Arg	Leu	Leu	Gly	Leu	Phe	Cys	Phe	Ala	Cys	Cys	Trp	
			580					585					590			
AAT	GAG	TGG	GCA	CCC	CCT	CTA	TTT	AGC	ATG	AAG	GAG	CCC	CAG	GCA	GGG	1824
Asn	Glu	Trp	Ala	Pro	Pro	Leu	Phe	Ser	Met	Lys	Glu	Pro	Gln	Ala	Gly	
		595					600					605				

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TAT	GCA	CAG	ACT	GAC	CAC	CAT	CCC	TCC	CCA	CCC	AGG	GTC	CAC	CCA	ACC	1872
Tyr	Ala	Gln	Thr	Asp	His	His	Pro	Ser	Pro	Pro	Arg	Val	His	Pro	Thr	
	610						615				620					
CGG	TGA	AGA	GAC	CAG	GAG	CAT	TGT	ACG	CAT	ACG	CGG	GTG	GTA	TTT	TTA	1920
Arg	*	Arg	Asp	Gln	Glu	His	Cys	Thr	His	Thr	Arg	Val	Val	Phe	Leu	
	625				630					635					640	
TGG	ACC	CCA	ATC	TGC	AAT	TCC	CAG	ACA	CCT	GGG	AAG	TGG	GAC	ATT	CTT	1968
Trp	Thr	Pro	Ile	Cys	Asn	Ser	Gln	Thr	Pro	Gly	Lys	Trp	Asp	Ile	Leu	
				645					650					655		
TGT	GTA	TTT	ATT	TTC	CTC	CCC	AGG	AGC	TGG	GGA	GTG	GTG	GGG	GGC	TGC	2016
Cys	Val	Phe	Ile	Phe	Leu	Pro	Arg	Ser	Trp	Gly	Val	Val	Gly	Gly	Cys	
			660					665					670			
AGG	TAC	GGT	TTA	GCA	TGT	GTT	TGG	TTC	TGG	GGG	TCT	CTC	CAG	CCT	TGT	2064
Arg	Tyr	Gly	Leu	Ala	Cys	Val	Trp	Phe	Trp	Gly	Ser	Leu	Gln	Pro	Cys	
		675					680					685				
TTT	GGG	CCA	AGT	TGG	AAC	CTC	TGG	CCC	TCC	AGC	TGG	TGA	CTA	TGA	ACT	2112
Phe	Gly	Pro	Ser	Trp	Asn	Leu	Trp	Pro	Ser	Ser	Trp	*	Leu	*	Thr	
	690					695					700					
CCA	GAC	CCC	TTC	GTG	CTC	CCC	GAC	GCC	TTC	CCC	TTG	CAT	CCT	GTG	TAA	2160
Pro	Asp	Pro	Phe	Val	Leu	Pro	Asp	Ala	Phe	Pro	Leu	His	Pro	Val	*	
	705				710					715					720	
CCA	TTT	CGT	TGG	GCC	CTC	CCA	AAA	CCT	ACA	CAT	AAA	ACA	TAC	AGG	AGG	2208
Pro	Phe	Arg	Trp	Ala	Leu	Pro	Lys	Pro	Thr	His	Lys	Thr	Tyr	Arg	Arg	
				725					730					735		
ACC	ATT	AAA	TTG	GC												2222
Thr	Ile	Lys	Leu													
			740													

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 740 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Ser	Val	Asp	Ser	Gln	Ser	Leu	His	Asp	Gln	Gln	Thr	His	Thr	Gln	Thr
1				5					10					15	
Ala	Ser	Gly	Gln	Ala	Leu	Lys	Gly	Asp	Gly	Asn	Leu	Tyr	Ser	Ser	Leu
			20					25					30		
Pro	Leu	Thr	Lys	Arg	Glu	Glu	Val	Glu	Lys	Leu	Leu	Asn	Gly	Asp	Thr
		35					40					45			
Trp	Arg	His	Leu	Ala	Gly	Glu	Leu	Gly	Tyr	Gln	Pro	Glu	His	Ile	Asp
	50					55					60				
Ser	Phe	Thr	His	Glu	Ala	Cys	Pro	Val	Arg	Ala	Leu	Leu	Ala	Ser	Trp
	65				70				75						80
Gly	Ala	Gln	Asp	Ser	Ala	Thr	Leu	Asp	Ala	Leu	Leu	Ala	Ala	Leu	Arg
				85					90					95	

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Arg	Ile	Gln	Arg	Ala	Asp	Ile	Val	Glu	Ser	Leu	Cys	Ser	Glu	Ser	Thr
			100					105					110		
Ala	Thr	Ser	Pro	Val	*	Thr	His	Arg	Leu	Gly	Ala	Pro	Val	Leu	Ser
		115					120					125			
His	Ile	Pro	Thr	Thr	Asp	Val	Leu	Ala	Ser	Pro	His	Arg	Ala	Ala	Pro
	130					135					140				
Ser	Pro	Ser	Gly	Met	Ala	Gln	Arg	Ser	Glu	Arg	Ser	Ile	Ser	Val	Gln
145					150					155					160
Gly	Leu	Cys	Val	Pro	Thr	Pro	Asp	Ser	Val	Ala	Ala	Pro	Glu	Gly	Ala
				165					170					175	
Leu	Ala	Ser	Asp	His	Pro	Leu	Leu	Ser	Lys	Arg	Glu	Arg	Glu	Asp	His
			180					185					190		
Pro	Ser	Leu	Thr	Cys	Ser	Ile	Ser	Ile	Ser	Gly	Leu	Ser	Phe	Leu	Ser
		195					200					205			
Thr	His	*	Leu	Cys	Gln	Ile	Trp	Gly	Phe	Asp	Thr	Arg	Arg	Arg	Glu
	210					215					220				
Arg	Gly	His	Pro	*	Asp	Ser	Gly	Gly	Thr	Glu	Glu	Pro	Glu	Pro	Trp
225					230					235					240
Thr	Pro	His	Cys	Glu	Pro	Glu	Asn	Lys	Gly	Arg	Gly	Ile	Val	Val	Gly
				245					250					255	
*	Thr	Phe	Leu	Ser	Pro	Ser	Leu	Leu	Pro	Ser	Gly	Gln	Arg	Arg	Gly
			260					265					270		
Leu	Arg	Thr	Tyr	Leu	Ser	*	Lys	Gln	Val	Trp	Asn	Pro	Ala	His	Thr
		275					280					285			
Ser	Leu	Ser	His	Thr	Gly	Trp	*	Asn	Pro	Glu	Lys	Gly	Arg	Asp	*
	290					295					300				
Pro	Arg	Pro	Pro	Asn	His	Arg	Lys	Asn	Lys	*	Arg	Leu	Ile	His	Ser
305				310						315					320
Val	Ser	Glu	*	Gly	Arg	Gln	Val	Cys	Leu	Leu	Thr	Gly	Met	Ala	*
				325					330					335	
Leu	Ser	Gly	Lys	Tyr	Leu	Glu	Ala	Met	Ser	Ala	Pro	Pro	Ser	Thr	Thr
			340					345					350		
Ser	Arg	Pro	Leu	Pro	Asn	Pro	Cys	Ala	Asp	Glu	Leu	Phe	Val	Gln	Gly
		355					360					365			
Leu	Val	His	Trp	Ser	Ile	Leu	Met	Glu	Ser	Ser	*	Gly	Leu	Arg	Leu
	370					375					380				
Ile	His	Lys	Ala	Phe	Val	Glu	Arg	*	Ile	Cys	*	Cys	Ala	His	Ser
385					390					395					400
Trp	His	Lys	Pro	Glu	Ala	Asn	Thr	Ala	Leu	Asn	Val	Ser	Pro	Arg	Gly
				405					410					415	
Gln	Glu	Pro	Arg	Thr	Pro	Thr	Pro	Gln	Ser	Asn	Thr	Ile	Leu	His	Tyr
			420					425					430		
Thr	His	Thr	His	Thr	His	Thr	His	Thr	His	Thr	His	Thr	His	Thr	Asp
		435					440					445			

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Ile Leu Leu Phe Ser Pro Trp Leu Phe Trp Gly * Asp * Ile Leu
 450 455 460
 Leu Gly Val Thr Ala Ser Glu Arg Ser Gly Gly Asp Arg Ala Glu Leu
 465 470 475 480
 His Gly Ala Val Phe Leu Ala Pro Gly Ser Gly Arg Pro Arg Met Thr
 485 490 495
 Ala Ser Glu Leu Val Ser Val Phe Gln Trp Pro Val Arg Gly Gly Asn
 500 505 510
 Ala Pro Thr Pro Pro Leu Leu Glu Ala Ala Pro Arg Arg Leu Gln Cys
 515 520 525
 Lys Arg Ala Asp Trp Cys Glu Asn Thr Arg Lys Ser Arg Cys Trp Pro
 530 535 540
 Cys Ser Leu Trp Gln Leu Ser Pro Gln Leu Gln Gly Pro Cys Lys Gly
 545 550 555 560
 Arg Ile Ser * Ala Arg Pro Gly Arg Gly Lys Arg Val Arg Phe Ser
 565 570 575
 Gly Ala Phe Ser Arg Leu Leu Gly Leu Phe Cys Phe Ala Cys Cys Trp
 580 585 590
 Asn Glu Trp Ala Pro Pro Leu Phe Ser Met Lys Glu Pro Gln Ala Gly
 595 600 605
 Tyr Ala Gln Thr Asp His His Pro Ser Pro Pro Arg Val His Pro Thr
 610 615 620
 Arg * Arg Asp Gln Glu His Cys Thr His Thr Arg Val Val Phe Leu
 625 630 635 640
 Trp Thr Pro Ile Cys Asn Ser Gln Thr Pro Gly Lys Trp Asp Ile Leu
 645 650 655
 Cys Val Phe Ile Phe Leu Pro Arg Ser Trp Gly Val Val Gly Gly Cys
 660 665 670
 Arg Tyr Gly Leu Ala Cys Val Trp Phe Trp Gly Ser Leu Gln Pro Cys
 675 680 685
 Phe Gly Pro Ser Trp Asn Leu Trp Pro Ser Ser Trp * Leu * Thr
 690 695 700
 Pro Asp Pro Phe Val Leu Pro Asp Ala Phe Pro Leu His Pro Val *
 705 710 715 720
 Pro Phe Arg Trp Ala Leu Pro Lys Pro Thr His Lys Thr Tyr Arg Arg
 725 730 735
 Thr Ile Lys Leu
 740

DATED this 6th day of October, 1998

THE WALTER AND ELIZA HALL INSTITUTE
 OF MEDICAL RESEARCH
 By Its Patent Attorneys
 DAVIES COLLISON CAVE

Fig 1

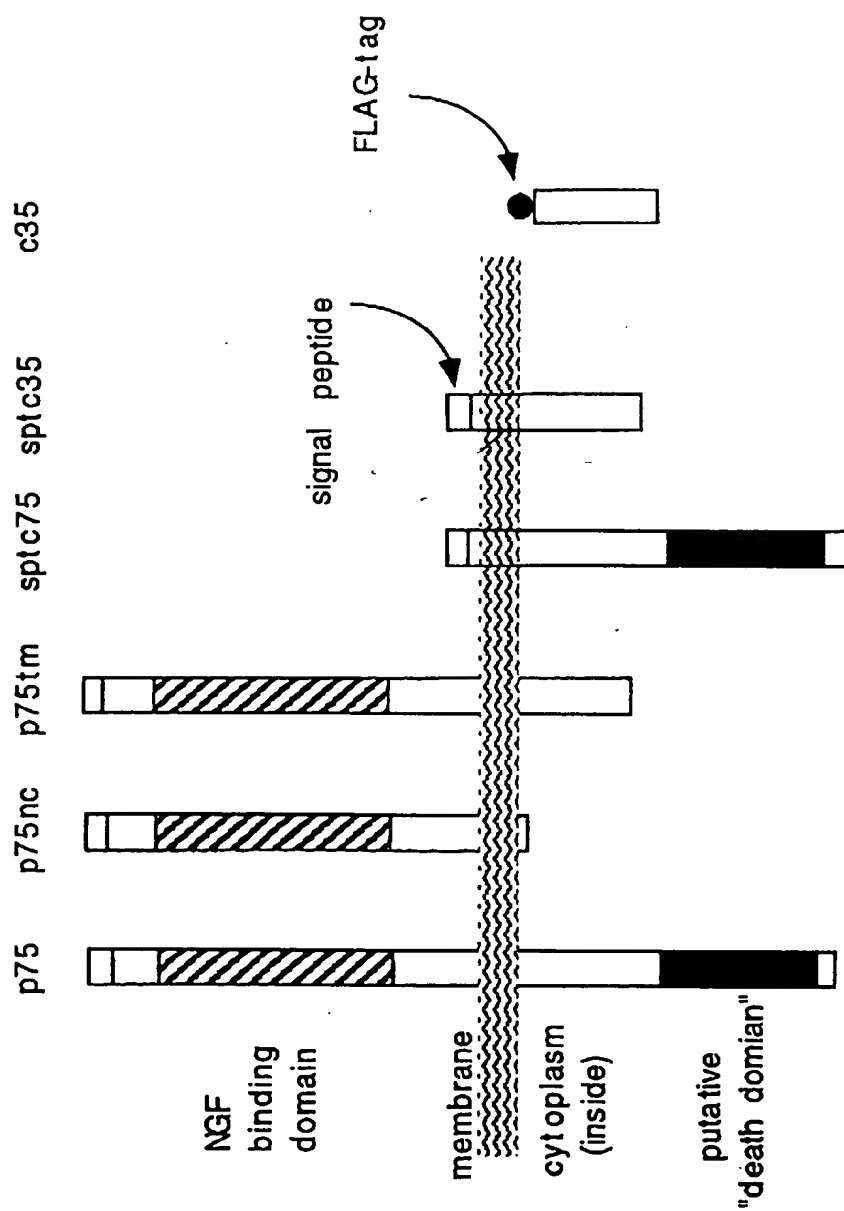
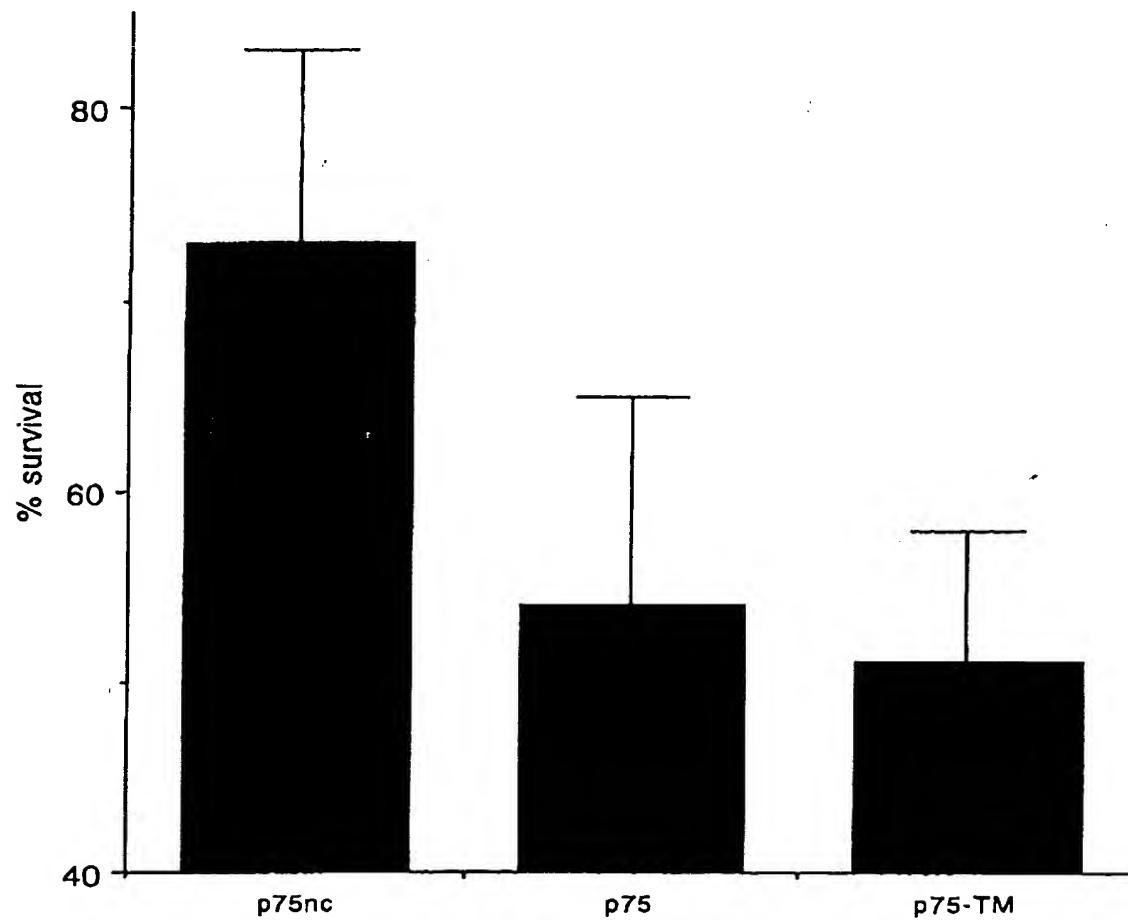


FIG 2

Survival of DRG neurons 17 hrs after microinjection, cultured in LIF



Expt S/U 11/11/97

p75nc.
73.3 ± 0.4

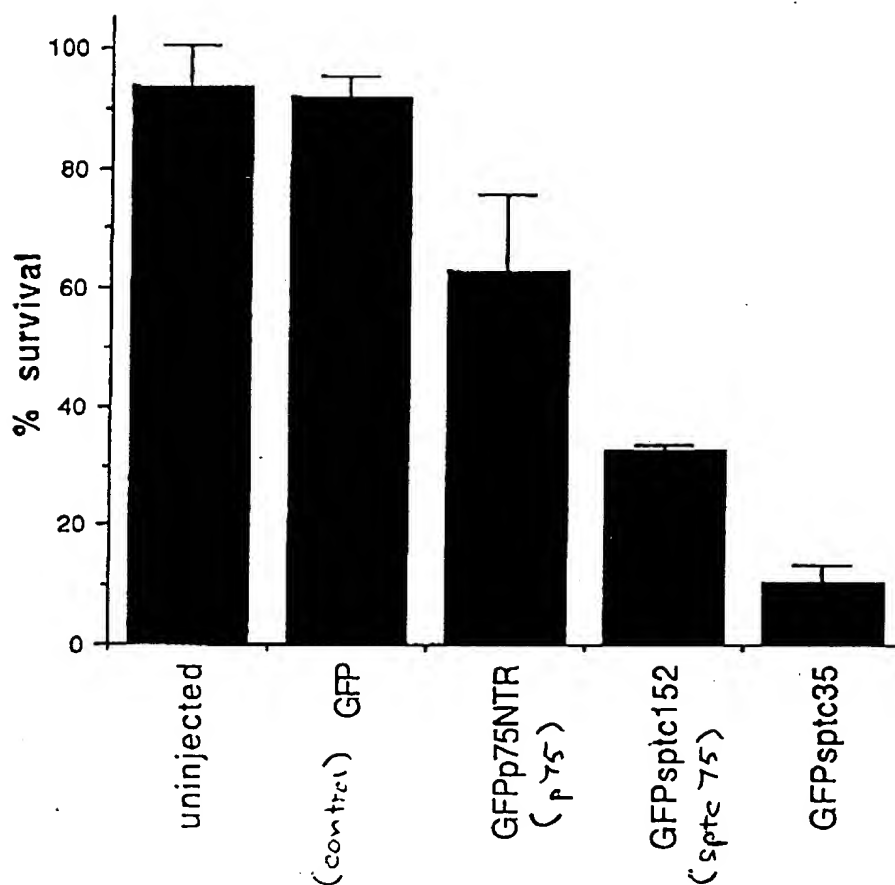
p75
64.4 ± 7

p75TM.
57.9 ± 0.6

expt S/U 4/11/97

FIG 3

DRG survival 16hr after microinjection,
cultured in LIF



GFP = the vector utilized

FIG 4

**C57 DRG survival 20 hr after mircoinjection,
cultured in LIF**

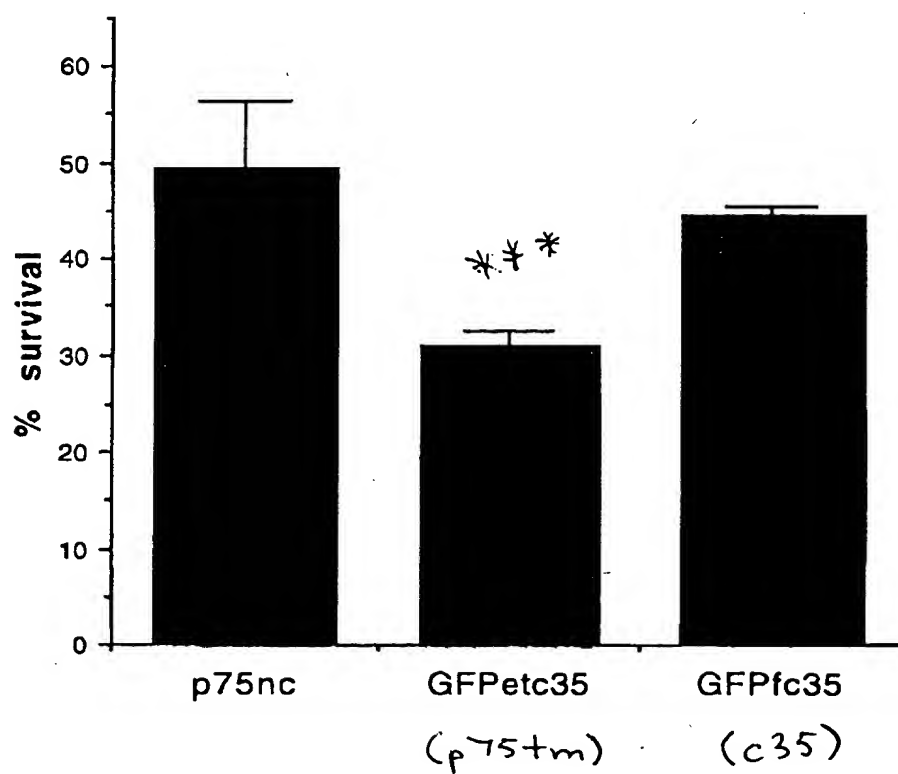


Fig 5.

